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SENSORY NERVES OF THE HUMAN HEART

EDWIN F. HIRSCH, M.D.
AND
JAMES F. ORME, M.D.
CHICAGO

FOR MANY years it has been known that the mammalian heart is supplied with both visceral efferent and visceral afferent nerves. Most of this information has been obtained by studying lower animals. The technical difficulties confronting investigations of the intracardiac distribution of the small nerve trunks and of the individual nerve fibers of the human heart probably account for the fact that there are relatively few studies of the nerves of this organ. The method most widely used to study nerve distribution in lower animals is that of staining with methylene blue as described by Ehrlich, or some modification of it. With this technic the best results are obtained by supravital staining or by staining of tissues immediately after the death of the animal. These conditions are difficult to meet in the postmortem examination of the human body. Various silver impregnation methods have been used, but these are seldom specific enough to differentiate individual nerve fibers from the surrounding connective tissue. With the present popular silver impregnation methods, such as that of Bielschowsky, a nerve fiber can scarcely be identified once it is separated from its trunk, because both nerve and connective tissue fibers are blackened. The silver impregnation method of Cajal as advocated by Nonidez [1] seems to offer some advantages of differentiating the various nerve fibers, particularly the parasympathetic and the sympathetic postganglionic fibers. The former are stained dark brown or black, and the latter yellow or orange, depending on the degree of impregnation. Also stained dark by this method are visceral afferent fibers and preganglionic parasympathetic fibers.

The large nerve trunks of the heart are readily identified and followed in serial sections stained by the usual methods; but these are not adequate for the study of the finer nerve trunks, the individual nerve fibers and the nerve endings. Nettleship [2] stated that Berkley

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From the Henry Baird Favill Laboratory, St. Luke's Hospital.

1. Nonidez, J. F.: Am. J. Anat. **65**:361, 1939.
2. Nettleship, W. A.: J. Comp. Neurol. **64**:115, 1936.

in 1894 was the first to suggest that there were sensory nerve endings in the heart. In 1895 Smirnow,[3] using a modification of Ehrlich's methylene blue technic, demonstrated a rich subendocardial plexus of afferent nerves in the auricles of dogs, cats and rabbits. The ventricles contained a similar, though less compact, plexus. A few years later Dogiel[4] demonstrated similar structures in the subepicardial tissues of the hearts of dogs, cats and a child. Woollard,[5] using a modification of Ehrlich's methylene blue stain, studied the efferent and afferent innervation of the heart in snakes, dogs, cats and rabbits. He found all the nonmuscular tissues of the heart to be richly innervated. This included the endocardium, the pericardium, the interstitial connective tissue and the valve structures. Because these tissues have no motor function he concluded that the nerves must be sensory in nature. Myelinated nerve fibers extended for a considerable distance in these structures, and as each fiber approached its termination it gradually lost its myelin sheath. The naked axis-cylinder then extended for a short distance and broke up into a complicated nerve ending. The actual ending was large and showed extensive subdivision, branching and rebranching until huge numbers of filaments were produced. Finally each filament ended in a bulbous expansion. Other observers,[6] using the same method, reported essentially similar afferent nerve endings. Some denied a sensory innervation, and Michailow, quoted by Nettleship,[2] proposed that the cardiac sensory nerve endings have capsules. Most observers have disagreed with this view.

Woollard,[5] Nettleship[2] and others have demonstrated that nerve trunks coming from the subepicardial region follow the course of the coronary arteries through the myocardium to the subendocardial plexus and often form an intricate network around the coronary arteries. Nettleship recognized three nerve plexuses in the heart: (1) those beneath the endocardium, (2) those in the adventitia of the aorta and in that of the pulmonary artery and (3) those in tissues surrounding the coronary arteries. Nettleship[2] investigated the distribution of the various components of the sympathetic and parasympathetic nerves of the heart in cats in which bilateral stellate and middle cervical sympathetic ganglionectomy, bilateral vagotomy, vagotomy proximal to the nodose ganglion or bilateral removal of the first five thoracic dorsal root ganglions had been performed. Following bilateral stellate and middle cervical ganglionectomy, the ventricular trunks and the epicardial network of the ventricles degenerated whereas similar structures of the auricles escaped. In addition, that portion of the endocardial

3. Smirnow, A.: Anat. Anz. **10**:737, 1895.
4. Dogiel, A. S.: Arch. f. mikr. Anat. **52**:44, 1898.
5. Woollard, H. H.: J. Anat. **160**:345, 1926; Heart **13**:319, 1926.
6. Nettleship.[2] Smirnow.[3] Dogiel.[4]

plexus near the apex of the ventricles, simple nerve endings in the fat and from one half to three fourths of the coronary artery plexus degenerated. The animals whose thoracic dorsal root ganglions had been removed had changes similar to those observed in animals which had undergone bilateral stellate and middle cervical sympathetic ganglionectomy except that the degeneration of the nerve trunks of the heart was much less. Some of the larger fibers of the coronary artery plexus and the portion of the endocardial plexus near the apex degenerated. With total vagotomy the greater part of the nerve fibers in the trunks of the epicardial plexus of the ventricles was spared. Atrial trunks had fragmentation and degeneration of the largest myelinated fibers. The endocardial, aortic and pulmonary artery plexuses similarly degenerated. With section of the vagus nerve proximal to the nodose ganglions, the myelinated fibers of the auricles were spared, but the fibers terminating about the epicardial ganglions degenerated.

Accordingly, in lower animals the stellate and cervical sympathetic ganglions supply the major portions of the efferent fibers to the epicardial network of the ventricles and to the portion of the endocardial plexus near the apex of the ventricles. The thoracic dorsal root ganglions supply afferent fibers to the ventricles and some of the myelinated fibers to the coronary arteries. The nodose ganglions of the vagus nerves supply afferent fibers to all of the endocardial plexus except the apical portion and to the aortic and pulmonary artery plexuses. The motor nucleus of the vagus nerve supplies the preganglionic fibers which are distributed to the epicardial ganglions.

MATERIAL AND METHODS

Blocks of tissue from the heart of 1 infant and 3 adults were studied. The infant tissue included portions of the left auricle and the left ventricle. All of the adult tissues were from the left ventricle along the course of the anterior descending branch of the left coronary artery. None of these blocks included tissue closer than 2 cm. from the origin of the artery mentioned. From one adult heart five serial blocks were taken, beginning at a level 2.5 cm. from the origin of the anterior descending branch of the left coronary artery. The first block was 1 cm. long as measured parallel to the coronary artery and about 1.5 by 1 cm. in the other dimensions. This block was fixed in formaldehyde solution, and sections cut from it by the freezing method were stained for myelin by the Spielmeyer method.[7] The second block was about 8 mm. long and was fixed in Bouin's fluid for seventy-two hours, after which it was embedded in celloidin (a concentrated preparation of pyroxylin) and paraffin in the manner suggested by Bensley and Bensley.[8] Serial sections, 8 microns thick, were cut

7. Mallory, F. B.: Pathological Technique, Philadelphia, W. B. Saunders Company, 1938.

8. Bensley, R. R., and Bensley, S. H.: Handbook of Histological and Cytological Technique Chicago, University of Chicago Press, 1938.

from this block, and every third slide was stained with the modification of the Masson-Goldner trichrome stain recommended by Foot.[9] When indicated, every slide was stained by this method or with the silver impregnation method of Bielschowsky.[7] The third block was cut about 3 mm. thick, was fixed in alcoholic chloral hydrate solution and was impregnated with silver according to the method described by Nonidez.[1] The fourth and fifth blocks corresponded in size and preparation to blocks 2 and 1, respectively. One block of tissue from an adult heart was fixed in Zenker's solution and embedded in paraffin, and serial sections, 8 microns thick, were stained with the trichrome method mentioned. Three blocks of tissue, about 2 mm. thick, from an adult heart were stained by a modification of Ehrlich's methylene blue method for nerve fibers.[7] These blocks were embedded

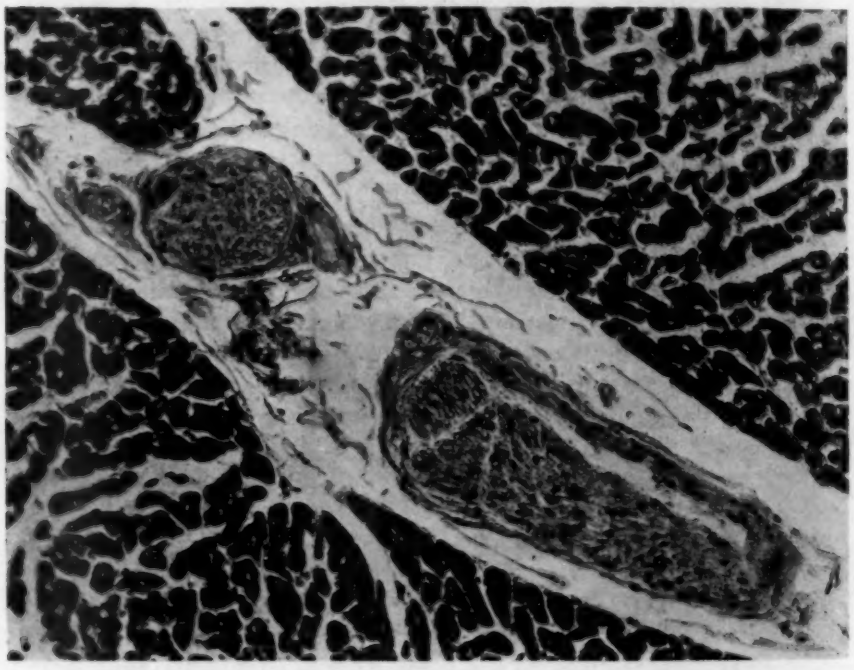

Fig. 1.—Photomicrograph of nerve trunks in the periarterial connective tissues between bundles of cardiac muscle. × 198.

in paraffin and sectioned. All sections were cut at right angles to the course of the coronary artery.

OBSERVATIONS

In the histologic sections prepared by each method, nerve trunks of varying sizes were readily identified and could be traced in serial sections through the block of tissue. The subepicardial regions had a large number of nerve trunks, many just beneath the serosal surface in the fibrous and fat tissues. The branches of the coronary artery were

9. Foot, N. C.: Pathology in Surgery, Philadelphia, J. B. Lippincott Company, 1945.

accompanied by nerve trunks of varying sizes, and these trunks accompanied the arteries through the myocardium to the subendocardial regions (fig. 1). Often more than one nerve trunk accompanied an artery, and the position of the nerve in relation to the artery varied. At times it was considerably removed and was in the loose areolar tissue, but at other levels it was in the compact fibrous adventitia of the artery. When in the latter position, the nerve trunk was usually small and seemed to end eventually in the adventitia of the artery. The nerve trunks often branched to follow the divisions of the artery, and sometimes a nerve trunk passed from an artery at almost a right angle,

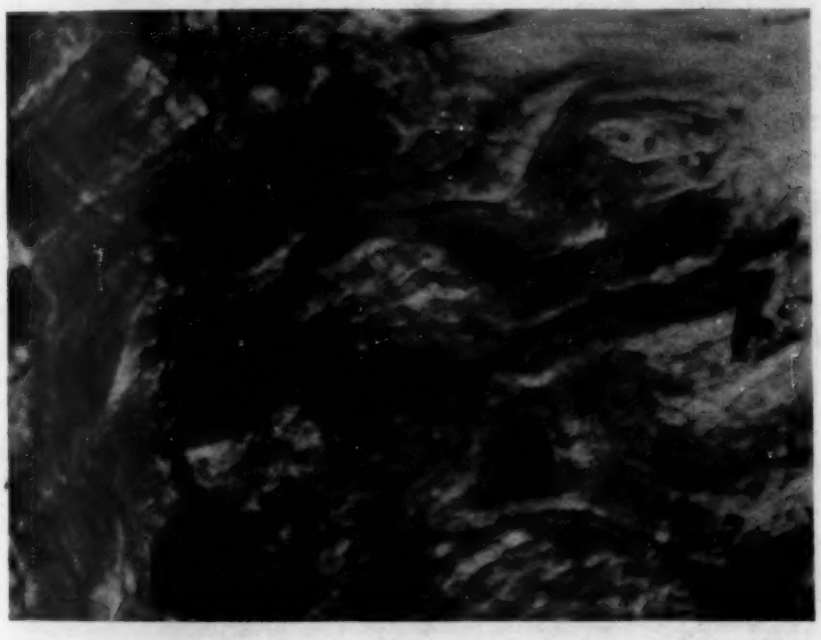

Fig. 2.—Photomicrograph of the terminal portion of a myelinated fiber in the adventitia of an artery. × 1360.

extended in loose areolar tissue between bundles of cardiac muscle and became associated with another blood vessel. The size of the nerve trunks gradually decreased toward the endocardium.

With sections prepared by freezing formaldehyde-fixed tissue and staining for myelin, nerve fibers with myelin sheaths were readily demonstrated in all blocks of tissue so prepared. The fibers varied in diameter, but most of them would be considered thick. Many had small varicose swellings along their course, and nodes of Ranvier were observed. Myelinated fibers were readily distinguished in nerve trunks and extended from the trunks into the surrounding stromal tissues or to

the adventitia of an artery, where they seemed to end. Myelin sheaths disappeared before the nerve terminated, but often a sheath could be traced into the adventitia of a blood vessel (fig. 2).

The silver impregnation method advocated by Nonidez [1] clearly demonstrated the nerve trunks. Most of the fibers were stained yellow, as were the surrounding fibrous and muscle tissues. A few large fibers were stained dark brown or black in the manner Nonidez ascribed to postganglionic parasympathetic or afferent nerve fibers (fig. 3). The heavily stained fibers extended from the nerve trunks into the surrounding connective tissue, but structures thought to be nerve endings as

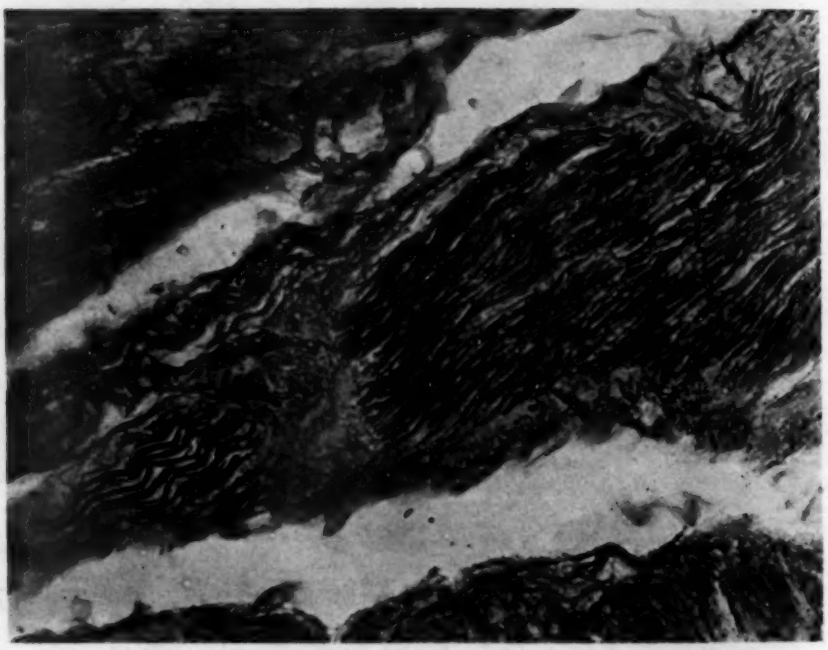

Fig. 3.—A nerve trunk with many myelinated fibers in perivascular connective tissues deep in the myocardium. × 346.

described by Nonidez [10] were not observed. A few sections of the tissues fixed in Bouin's solution were stained according to the Bielschowsky method, but this was abandoned because individual nerve fibers could not be distinguished from the impregnated reticulum.

Nerve ganglion cells were observed in the epicardial tissues of the auricle of the infant's heart, but none was observed in the tissues of the ventricle. This is in agreement with the studies made by Woollard [5] and Nettleship [2] in lower animals but is in contrast to the studies of

10. Nonidez, J. F.: Am. Heart J. **26**:577, 1943.

animal tissues made by Dogiel and Smirnow. In the limited investigation with the methylene blue technic, the tissues stained irregularly, and while nerve trunks and nerve fibers could be identified, nerve endings of the type described were not observed. The same is true of the silver impregnation method of Nonidez.[1] Small clusters of black silver compounds were distributed in the tissues at some levels, but these were not definite enough to be considered sensory nerve endings. Encapsulated nerve endings were not observed in any of the tissues. This is in agreement with the studies made in lower animals by Nettleship.[2]

The presence of myelinated nerve fibers in the heart in regions not requiring motor function, as well as their structure, indicates that they are afferent. Woollard [5] has described myelinated postganglionic parasympathetic fibers arising from the intracardiac ganglions. However, most of these fibers lose their myelin sheaths after a short course and are distributed to the musculature and the conduction system of the auricles. Kuntz [11] stated that some gray rami communicantes of the sympathetic system contain myelinated fibers. However, given a myelinated nerve fiber of the ventricle of the heart which extends into and seems to end in fat or fibrous connective tissue, the assumption is reasonable that such a fiber is afferent in function. Such fibers are widely distributed in the subepicardial and subendocardial tissues and in the perivascular connective tissues of the myocardium.

COMMENT

Many studies of the distribution of the nerves of the hearts of lower animals have been made. In some, portions of the nerve supply have been interrupted, and the subsequent areas of degeneration indicated specific nerve supplies of various regions of the heart. Such studies of the nerve supply of the human heart are not feasible. The anatomic relations of the sensory nerves of the human heart have great interest, because these fibers are the medium for transmission of the pain sensations that occur with various forms of heart disease, notably angina pectoris, coronary occlusion and acute myocardial infarction. However, only a few reports of such studies of the human heart have been published. According to Gorham,[12] the myocardium itself is insensitive, but the heart as a structure has numerous afferent (sensory) fibers that respond to painful stimuli. These fibers are in the adventitial layer of the coronary arteries and send terminal branches at intervals to the smooth muscle of the media. This sensory nerve system, Gorham stated,

11. Kuntz, A.: The Autonomic Nervous System, Philadelphia, Lea & Febiger, 1945, pp. 48-49.

12. Gorham, L. W.: A. Research Nerv. & Ment. Dis., Proc. (1942) **23**:337, 1943.

is basic in any discussion of the mechanism of cardiac pain. The pain-receptive structures in other parts of the body (cornea, Tower [13]; skin, Gasser [14]) are nerve fibers, a large portion myelinated but losing their sheaths in the terminal branches. Gasser observed both myelinated and nonmyelinated fibers in sensory nerves, the proportion of each ranging widely.

The myelinated fibers observed in nerves distributed along the coronary arteries of the heart are considered to be afferent and hence sensory in function. No conclusions are possible as to whether these afferent nerve fibers are part of the vagus nerve, with their cell bodies in the nodose ganglions, or whether they accompany the sympathetic nerves and have their cell bodies in the dorsal root ganglions of the spinal cord. These visceral afferent (sensory) fibers in the nerves of the human heart are the anatomic structures by which sensory impulses are transmitted to the central nervous system, and, as Gorham [12] has stated, they are basic in any discussion of the mechanism of cardiac pain. If a theoretic nonmedullated sensory fiber component, not determined by these anatomic studies, is present, the arterial and periarterial tissues could have a much larger sensory supply than the number of myelinated fibers would indicate. Our study does not determine which tissues of the ventricle of the human heart have the largest supply of afferent nerves, but other reports state that the base of the heart has the richest supply of nerves.

The changes of atherosclerosis are usually in the first portion of the coronary arteries, that is, at the base of the heart, where the nerve supply is greatest. The arteriosclerotic changes occurring in the wall of the artery seem not to initiate the painful sensations associated with coronary disease, at least not the severe form. Although the exact nature of the stimulus producing pain in these sensory nerves of the heart is not fully understood, by general agreement this stimulus seems to be associated in some way with impedance or suppression of the coronary blood flow. A popular view holds that the pain is caused by relative ischemia or anoxemia of the cardiac muscle. If the studies which indicate that the myocardium itself is insensible to painful stimuli are valid, this explanation of the origin of the painful sensation has little to support it anatomically except in an indirect way. Lewis [15] has proposed that the ischemic muscle liberates a factor, "P," which diffuses into the tissues and there stimulates the nerve endings. Others have proposed that acid metabolites, such as lactic

13. Tower, S. S.: A. Research Nerv. & Ment. Dis., Proc. (1942) **23**:16, 1943.

14. Gasser, H. S.: A. Research Nerv. & Ment. Dis., Proc. (1942) **23**:44, 1943.

15. Lewis, T.: Arch. Int. Med. **49**:713, 1932.

acid, which accumulate during ischemia provide the stimulus for the sensation of pain. Moore and Singleton [16] observed a pain response in dogs when lactic acid was injected into the coronary circulation, and have formed a theory concerning cardiac pain because a concentration of lactic acid approaching a pain-producing threshold could be demonstrated in blood flowing from the coronary sinus with fatigue of the heart muscle. Moore and Greenberg [17] emphasized lactic acid as the tissue factor stimulating painful sensations in circulatory disturbances of the heart. Doubtless small concentrations of many other definite chemical substances injected into the coronary circulation would cause similar painful sensations.

The myelinated nerves distributed in the fibrous tissues along the coronary arteries and the terminal branches extending into the walls of these vessels suggest, from anatomic considerations, that stimuli for pain develop in the tissues in and about these arteries. Katz, Mayne and Weinstein [18] studied ischemia as a cause of pain experimentally in dogs and concluded that the sensory response is due not to occlusion of the coronary artery and interference with the intra-arterial flow of blood but to direct stimulation of afferent fibers in the nerve plexus surrounding the coronary vessels. Gorham [12] produced cardiac pain in dogs by making tension on the coronary arteries without interfering with the circulating blood, that is, ischemia. These results led him to suggest that tension of the tissues in and about the coronary arteries is the stimulus for cardiac pain. Supporting evidence is derived from the observation that occlusion of the lumen of a normal peripheral artery, such as a femoral artery, is associated with pain and that when the obstruction is sudden the pain is severe and at the site of obstruction. The excruciating pain associated with the tearing of the tissues by a dissecting aneurysm of the aorta is well known and is a diagnostic clinical symptom. Here the trauma done to tissues rather than anoxemia is regarded as the stimulus of the pain, and more clearly we realize that injuries of the arterial tissues cause pain and that, as in other parts of the arterial system, the aorta and the arteries (or veins) have nerves with sensory fibers. Woollard's studies of the innervation of peripheral blood vessels led him to comment that the endings of myelinated nerves (sensory) have been observed in the walls of blood vessels by many authors but that these observations seem to have gained little credence, being either ignored in the interpretation of vascular disturbances or, if admitted, regarded as subserving only a

16. Moore, R. M., and Singleton, A. O.: Proc. Soc. Exper. Biol. & Med. **32**:1492, 1935.

17. Moore, R. M., and Greenberg, M. M.: Am. J. Physiol. **118**:217, 1937.

18. Katz, L. N.; Mayne, W., and Weinstein, W.: Arch. Int. Med. **55**:760, 1935.

minor sensory function. As early as 1905, according to Woollard,[8] Lapinsky described five types of nerve endings of medullated nerves in blood vessels: (1) those of myelinated fibers which lose their sheaths at some distance from their termination, to end finally in freely branched twigs; (2) branched endings forming a terminal brush of considerable length; (3) bulbous thickenings of the ends of small twigs given off at right angles from medullated nerves; (4) small fibers ending abruptly in a granule; (5) undetermined forms, in which branches of medullated nerves appear to end in large oval swellings. Woollard, from his own observations on the endings of medullated (sensory) nerves in and about blood vessels, stated that some are pacinian corpuscles, while others end freely in adjacent fat and connective tissues and in long, extended twigs in the adventitia of the vessels. Woollard observed that medullated fibers were most abundant on the peripheral vessels and on this basis concluded that puncture or ligation of these was more likely to be painful than that of large vessels. Gammon and Bronk[19] observed the initiation of afferent impulses in sensory nerves of the mesentery, which increased in number and frequency with distention of the mesenteric blood vessels at pressures above a critical level. A decrease of the caliber of the vessels caused a decrease of the afferent impulses.

These references to the sensory innervation of systemic arteries do not include all pertinent reports. Studies of the distribution of the sensory nerves of the heart demonstrate that here, also, the fibers are arranged in close association with the coronary arteries. This pattern of sensory nerve supply common to the peripheral and the coronary arteries, with perhaps some specific adaptations in the heart, provides an anatomic structure in which comparable principles may be noted. There is no dispute concerning the fact that various physical or chemical stimuli can arouse painful sensations in tissues; the recognition of the specific component with each stimulus depends on how minute the analysis can be. Sudden occlusions of peripheral arteries are painful by common clinical observation, whether caused by tension or actual injury of the tissues. No one thinks of this immediately painful experience in the general terms of anoxemia. Coronary arteries, as disclosed by anatomic studies, have a sensory nerve supply similar to that of the peripheral arteries. Accordingly, in each category painful sensations can occur with comparable stimuli. Since in the heart the sensory nerves are distributed mainly along the coronary arteries, and thrombosis usually occurs in the proximal segments of these arteries, the stimuli producing cardiac pain in vascular accidents of the heart would seem to arise, at least from anatomic considerations, not in the

19. Gammon, G. O., and Bronk, D. W.: Am. J. Physiol. **114**:77, 1935.

myocardium but in the arterial and periarterial tissues and in a manner entirely like that noted with sudden thrombosis of a peripheral artery. This view of the site of origin of the pain attending cardiac disease may seem novel, but actually it brings present concepts of the origin of the painful cardiac sensations of coronary disease and other cardiac disorders into a common pattern, applicable with comparable injuries or stress to both the coronary and the peripheral arteries. This concept also focuses attention not on the heart as a unit structure but on its sensory components and their significant relation to the coronary arteries.

SUMMARY

Myelinated (sensory) nerve fibers are distributed along the coronary arteries and their branches in the myocardium. Their terminal divisions are filaments without myelin sheaths, some of which extend directly into the walls of the arteries.

These sensory fibers are the anatomic pathway by which painful sensations arising in the heart are transmitted to the central nervous system.

The observation that these fibers are distributed along and in the walls of the coronary arteries suggests that in the heart the painful stimuli arise in and about the coronary arteries rather than in the muscle tissues.

Sensory fibers are distributed to the walls of peripheral arteries in a pattern quite similar to that observed with the coronary arteries of the heart. Accordingly, this common pattern of distribution of the sensory nerves of both the coronary and the peripheral arteries provides a common anatomic basis for the reception of pain sensations aroused by comparable conditions in all arteries of the body.

The tissue factors arousing the pain sensation associated with an injury or with thrombosis are similar for all arteries, the response depending on the sensory nerve supply.

FOCAL ANEMIA, LEUKOCYTOSIS AND FATTY INFILTRATION OF THE LIVER (SO-CALLED SEPTIC LIVER SPOTS)

FRANZ WENGER, M.D.
MARACAIBO, VENEZUELA

EVERY pathologist is familiar with certain yellowish spots frequently seen on the surface of the liver, which stand out clearly against the surrounding tissue. For the first time, this lesion was mentioned briefly in 1904, by Beneke,[1] who stated that in cases of acute lesions of the brain, acute peritonitis and other diseases accompanied by shock there occur in the liver characteristic wedge-shaped anemic foci; he explained them as anemia due to vasomotor spasm occurring during agony. Five years later Helly [2] gave a thorough macroscopic description. He distinguished two kinds of liver spots: The first are somewhat diffuse and always superficial, and their shape and localization indicate their origin—a postmortem change due to pressure of the ribs or of the abdominal viscera. The second have sharp, although irregular, outlines and are grouped around the branches of the portal vein. By microscopic observation of this second group of liver spots he found basophilia of the hepatic cells (in sections stained with methylene blue and eosin), focal edema and anemia, and he explained the striking yellowish color of the lesions as being due to diffuse fatty degeneration of the whole organ, which inside the spot might be more visible because of the anemia. Helly expressed the belief that the focal edema was prior to the focal anemia, which in his opinion is produced by the compression of the sinusoids, perhaps through cloudy swelling of the liver cells. At any rate, he said, the cause of the lesion must be looked for in toxic matters, circulating in the blood during acute sepsis; for that reason, and because he had found the spots only in cases of sepsis, he called them "septic spots."

More recently Roessle,[3] speaking of hepatitis in general, considered these spots as localized serous hepatitis and stated that no sharp limit can be drawn between the two forms of serous hepatitis, the localized

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From the Department of Pathology, Tumor Clinic.

1. Beneke, R.: Deutsche med. Wchnschr. **30**:1489, 1904.
2. Helly, K.: Verhandl. d. deutsch. path. Gesellsch. **13**:312, 1909.
3. Roessle, R.: Schweiz. med. Wchnschr. **10**:4, 1929.

and the diffuse. On the other hand, Gerlach [4] found the "spots" on the surface and deep in the parenchyma, and they were well pronounced in cases of sepsis, especially after abortion; but he saw them also in nonseptic conditions, sometimes associated with cerebral lesions.

The references cited are, as far as I can see, all that are to be found in the literature on this subject—which seems to have been badly neglected. This neglect is the more astonishing even in view of the fact that the descriptions given in the foregoing paragraph are quite inadequate in the light of my experience, especially in regard to the microscopic structure; only to Helly's segregation of spots due to postmortem compression should everybody fully agree.

From my material of 32 cases, used for the following report, such postmortem changes were carefully excluded. Among the causes of death in these cases there was a slight prevalence of acute peritonitis, sepsis and cerebral lesions as compared with the frequency of the diseases in this autopsy population. During this investigation two types of lesions, quite different, especially in their microscopic appearance, were found to occur, which formerly certainly have been listed together as "septic liver spots." They will be described separately as types A and B.

GROSS APPEARANCE

Type A (26 cases).—The lesions of type A were found nearly always on the anterior surface of the liver, more frequently on that of the right lobe than on that of the left, and generally near to the insertion of the ligamentum falciforme. The size of these spots varied greatly, from 5 mm. to 7 cm. in diameter. Their shape usually appeared to be irregularly polygonal. The color was yellowish and always clearer than the rest of the liver surface. The limits were sharp all around, or were sharp on one side and shaded away into the rest of the surface on the other. Frequently the sharp limit was produced by a dark zone of hyperemia around the spot. On the cut surface the parenchyma beneath the spot was also yellowish; the depth of this clear zone rarely exceeded 1 cm.; only exceptionally it was wedge shaped, its form being generally quite irregularly flat. Some livers showed several spots of the type here described.

Type B (6 cases).—In 4 cases the spots were exactly on the inferior border of the liver, nearly in the notch where the round ligament inserts, whereas in a fifth there was a spot on the under surface. They were regularly round and 1 cm. in diameter. There was no hyperemia of the surrounding tissue. On the cut surface they were wedge shaped, and a big portal space could be distinguished on the point of the wedge. One lesion of this type was associated with another round spot on the under surface of the liver, where a fibrous adhesion inserted, coming from the gallbladder. On the cut surface the part of the parenchyma belonging to this second spot was not wedge shaped but merely flat. In the last

4. Gerlach, W., in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1930, vol. 5, pt. 1, p. 103.

case of this group the under surface of the liver showed several clear spots, very irregular, measuring up to 5 mm. in diameter, like splashes.

MICROSCOPIC APPEARANCE

Type A.—The sharp limits of the clear areas which were so conspicuous to the naked eye frequently disappeared after the staining was done, and I used to confront the stained slides with the paraffin blocks to get oriented quickly. Orientation was easier when the hyperemia of the margins was marked. The common feature of all the clear areas of hepatic parenchyma which belonged to this group was the anemia, with the sinusoids practically devoid of red cells. In the cases of anemia alone the sinusoids were empty or contained some slightly eosinophilic amorphous matter, apparently coagulated albuminous fluid. In some cases this eosinophilic matter was found also outside the sinusoids, in the spaces of Dissé. One case showed the sinusoids under the liver spot filled by slightly eosinophilic round corpuscles, which contrasted strongly with the bright red color of normal red blood cells; they were probably red cells from which the hemoglobin had been extracted (by stasis?).

In only 3 cases, in which the liver in general was more or less normal, were all the signs of Roessle's serous hepatitis visible beneath the clear spot, i. e., edema fluid in the spaces of Dissé, dissociation of hepatic cell cords, necrosis of hepatic cells, especially around the central veins and others. Usually, when serous hepatitis was found, none of the signs of injury was more pronounced inside the clear area than in the rest of the parenchyma. Yet the local anemia seemed to make more visible such damage of tissue, so that it stood out more clearly in the slide, and at a rapid examination the anemic area feigned a more severe injury when, as a matter of fact, the same degree of injury was present in the whole organ. Fatty infiltration was never so prominent as to be responsible for the clear color of the area, but in many cases it was heavier under the clear spot than elsewhere. I could never find Helly's basophilia of the protoplasm of the hepatic cells, even when employing Mallory's eosin and methylene blue stain.

In 11 cases the leukocytosis of the area was striking, the area contrasting in this respect with the rest of the parenchyma, where leukocytes were found with no more frequency than usual. In these cases the sinusoids were crammed with polymorphonuclear leukocytes, lymphocytes and large mononuclear cells, and such cells were found also outside in the pericapillary spaces. This dense leukocytosis was sometimes so well pronounced that practically all the sinusoids of the clear area were filled with white blood cells, just as they are being filled with red cells (fig. 1). In other cases the leukocytosis was more patchy in distribution, being localized especially under Glisson's capsule and in the peripheral zone of the clear areas, and here the leukocytic sinusoids were sometimes the immediate continuation of the sinusoids of the hyperemic margin, which were crowded with erythrocytes (fig. 2). Sometimes a case was classified at first as one simply of anemic or of "serous" hepatitis, and only after cutting several blocks of tissue a small leukocytic area could be found. It is noteworthy that this leukocytosis of the sinusoids was not at all related to injury of liver cells (fig. 3) or other signs of hepatitis, being seen in livers otherwise practically normal, as well as in livers showing severe damage. Outside the clear area I could never find sinusoids crowded to such a degree by leukocytes.

Type B.—Just as in their gross appearance, the lesions which belonged to this group were quite uniform in their histologic structure. Their limits were perfectly

sharp and ran more or less along the portal spaces. The liver cells were infiltrated by big droplets of fat, which filled the whole body of the cell; only around the portal spaces some few hepatic cells without fat could be found (figs. 4 and 5). The whole area was rather anemic, but there was no marked leukocytosis. In 1 case a few minute foci of necrosis were seen, with secondary leukocytic infiltration. In another case the fatty infiltration was produced by tiny droplets, and not by big ones. The branches of the portal vein and of the hepatic artery which ran toward these areas of fatty infiltration appeared to be normal.

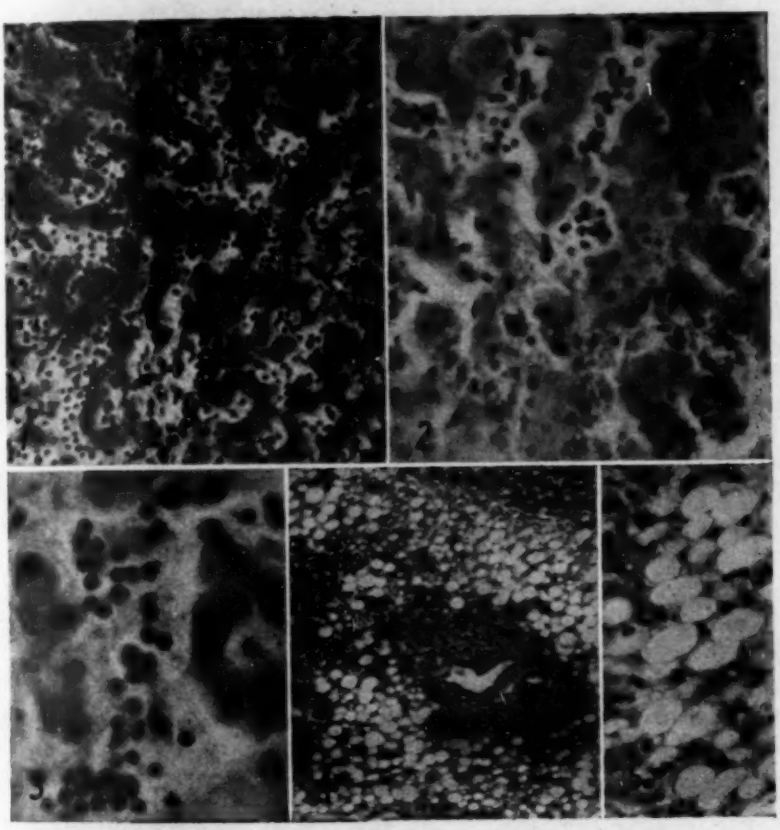

Fig. 1.—Focal leukocytosis of the liver. × 180.

Fig. 2.—Border of leukocytic focus; sinusoids are shown, which are filled on one side (top) by leukocytes and on the other side (bottom) by red blood cells (belonging to the hyperemic margin). × 365.

Fig. 3.—White blood cells filling the sinusoids; the lining hepatic cells are normal. × 690.

Fig. 4.—Focus of fatty infiltration, sharply limited against the adjoining normal liver tissue. × 24.

Fig. 5.—Liver cells heavily infiltrated by big fat droplets inside the focus. × 330.

COMMENT

The findings in the lesions which generally are called "septic spots" are both surprising and difficult to explain. In the cases of type A the most prominent and only common feature was the severe local anemia, which certainly determined by itself the clear color of the area. As pointed out already by Beneke,[1] such severe anemia is incompatible with life, and as there is no necrosis, the anemia cannot have started before agony. As no obstruction ever was found in the pertinent blood vessels, a spastic contraction of these vessels is supposed to exist, although it should be remembered that two different sets of vessels, the branches of the hepatic artery and those of the portal vein, have to close their blood supply simultaneously. Still little is known about the finer structure and function of the circulatory system of the liver; only recently Freerksen[5] and especially Coronini[6] complemented Pfuhl's[7] earlier report of particular musculoelastic structures in the liver arterioles which certainly regulate the blood pressure in the capillaries for proper mixture of arterial and venous blood. Transitory dysfunction of such a mechanism might result in the local anemia here described.

Roessle's[3] view that the "septic spots" represent just local serous hepatitis is based principally on the findings of edema fluid in Dissé's spaces; this, as well as the other signs of serous hepatitis, was only rarely seen in any significant degree in the foci here described, so as to contrast with the remainder of the parenchyma. And where such a difference is seen between the liver structure inside and that outside the clear area, it is explained more easily as an injury from prolonged anemia, occurring perhaps when agony lasted sufficiently long to permit the development of such changes.

To find dense accumulations of leukocytes in these areas was most surprising. Of course, the suggestion that this must be a severe inflammation was near at hand, but had to be rejected. In diffuse hepatitis, such as that in catarrhal jaundice (infectious jaundice), leukocytic infiltration is not conspicuous,[8] although some authors[9] have mentioned it.

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5. Freerksen, E.: Klin. Wchnschr. **22**:733, 1943.

6. Coronini, C.: Zentralbl. f. allg. Path. u. path. Anat. **82**:241, 1944.

7. Pfuhl, W., in Möllendorf, W.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1932, vol. 5, pt. 2, p. 235; cited by Coronini.[6]

8. (*a*) Gaskell, J. F.: J. Path. & Bact. **36**:257, 1933. (*b*) Barber, H., and Osborn, G. R.: ibid. **49**:581, 1939. (*c*) Siegmund, H.: München. med. Wchnschr. **89**:463, 1942.

9. (*a*) Klemperer, P.; Killian, J. A., and Heyd, C. G.: Arch. Path. **2**:631, 1926. (*b*) Schrumpf, A.: Ann. d'anat. path. **9**: 17, 1932. (*c*) Roholm, K., and Iversen, P.: Acta path. et microbiol. Scandinav. **16**:427, 1939. (*d*) Roulet, F.: Virchows Arch. f. path. Anat. **310**:436, 1943. (*e*) Lucké, B.: Am. J. Path. **20**: 471, 1944. (*f*) Roessle.[3]

Wohlwill [10] described a form of diffuse hepatitis which was accompanied from the beginning by leukocytic infiltration just as any other inflammation elsewhere in the body. The closest approach to my findings was that presented by Dible, McMichael and Sherlock,[11] who in cases of hepatitis of different causes saw in the sinusoids a general enlargement and hyperplasia of "endothelial" cells, giving the hepatic lobule an abnormally cellular appearance; they even described small focal accumulations of cells, including leukocytes; but this as well as all the other reports relate the cell accumulations to degeneration or necrosis of liver cells, whereas in several of my cases the liver cells seemed to be perfectly normal, or cell degeneration was in no relation in degree or localization to leukocytosis. Bacterial stains were always negative. Moreover, the number of cells inside and outside the sinusoids exceeded very much that of all the reports cited in the foregoing pages.

It is for the moment impossible to give a satisfactory explanation for this curious local leukocytosis. But I wish to point out that the leukocyte content of the liver, as that of some other organs, is known to vary greatly and to be quite independent of inflammation and of the leukocyte content of the peripheral blood. Gräff [12] described capillaries which were completely filled with leukocytes, with only occasional red blood cells between them, and he supposed that such accumulations of leukocytes in the inner organs may even influence inversely the leukocyte content of the peripheral blood. Hino,[13] after extensive anatomic studies, concluded that the only possible cause for this accumulation of leukocytes is the number and the length of the capillaries of some inner organs, especially of the liver, the kidney and the spleen, and that the leukocytes adhere to the vessel walls because of the slowing down of the blood stream. In my cases of severe local anemia such slowing down must have been marked, and if Hino's interpretation of the general leukocytosis of inner organs is correct, the local accumulation of white cells in anemic areas should be simply mechanical, due to the stasis, and without any relation to chemotaxis or similar phenomena.

While the simply anemic and the leukocytic foci of type A seemed not to be fundamentally different lesions, as there were borderline conditions of only limited leukocytic infiltration, the lesions of type B, the fatty infiltrations, constituted a second group, totally different from the first, and there was no bridge between them. These fatty foci have certainly a different causation and pathogenesis, and their shape and the close relation (macroscopic and microscopic) to the bigger branches of blood vessels point clearly to an embolic origin, although this investiga-

10. Wohlwill, F.: Schweiz. Ztschr. f. allg. Path. u. Bakt. **2**:240, 1939.
11. Dible, J. H.; McMichael, J., and Sherlock, S. P. V.: Lancet **2**:402, 1943.
12. Gräff, S.: Berl. klin. Wchnschr. **58**:84, 1921.
13. Hino, J.. Virchows Arch. f. path. Anat. **256**:30, 1925.

tion could not disclose the nature of the emboli. Very similar foci of severe local fatty infiltration were observed by Menkin [14] in dogs after intravenous injection of necrosin, a substance contained in the euglobulin fraction of purulent exudate, which seems to be responsible for the tissue damage in typical acute inflammations. I could not find any other reference to focal fatty infiltration of the type here described.

The name given to these clear areas by Helly,[2] which still is widely in use, may safely be abandoned. First, these lesions are not spots; they are tridimensional. Second, there exists no relation to septic diseases, as they occur in many nonseptic diseases. Further studies are necessary to cast light on their causation and pathogenesis. In the meantime, purely descriptive names should be used, which take into account the histologic aspects of the lesions, such as "focus of anemia" and "focus of fatty infiltration," respectively.

SUMMARY

In a series of cases of so-called septic spots of the liver, two quite different types of lesions were observed. In the first the parenchyma under the spot was severely anemic, this lack of red blood cells being responsible for the clear color of the whole area. In many cases there was in addition to the anemia a dense accumulation of polymorphonuclear leukocytes, lymphocytes and large mononuclear cells inside and outside the sinusoids.

The second type, in most instances, was wedge shaped on the cut surface and sharply limited; it showed severe fatty infiltration of the liver cells.

No satisfactory explanation can be given as to the causation and genesis; anyhow, an inflammatory origin can be ruled out probably for both groups of lesions; in regard to the first, all the findings, including the local leukocytosis, might be explained by vascular spasm and consecutive slowing down of the blood stream during agony. The second group seems to have an embolic origin. As both types of lesions are associated with a great variety of diseases, the name "septic spots" should be substituted by "foci of anemia" or "foci of fatty infiltration," respectively.

14. Menkin, V.: Arch. Path. **36**:269, 1943.

PATHOGENICITY OF BAGASSE

B. GERSTL, M.D.
HARTFORD, CONN.

M. TAGER, M.D.
NEW HAVEN, CONN.
AND
N. A. MARINARO, M.D.
HARTFORD, CONN.

SUGAR CANE from which the sugar content has been extracted is called bagasse. This material is stored in the open for months and even years; then it is broken, processed and finally pressed into various shapes yielding building and insulating boards, such as "celotex." Workers breaking bagasse sometimes acquire a respiratory disease characterized clinically by cough, dyspnea, occasional hemoptysis, night sweats, chills and intermittent low fever. Roentgenologic examination of the chest shows miliary mottling throughout both lungs in almost all cases. Most of the patients recover after prolonged illness. Although extensive clinical studies have been carried out, and in 1 case autopsy,[1] uncertainty still prevails as to the genesis of this disease.

In the opinion of Sodeman and Pullen [2] the etiologic agent and the mechanism of the changes are obscure, while Jamison and Hopkins [3] have expressed the belief that micro-organisms, probably fungi, growing on and causing deterioration of the fibers, and inhaled with these, cause the disease. Castleden and Hamilton-Paterson,[4] on the basis of positive cutaneous reactions obtained with extracts of bagasse, interpreted the disease as an allergic reaction. LeMone, Scott, Moore and Koven [5] in a recent report attributed the changes to the high silica content of the bagasse fiber.

From the Central Laboratory of Pathology and Research, State Tuberculosis Commission, Hartford, Conn. (Dr. Gerstl) and the Department of Bacteriology, Yale University, New Haven, Conn. (Dr. Tager)

1. Sodeman, W. A., and Pullen, R. L.: New Orleans M. & S. J. **95**:558,1943.
2. Sodeman, W. A., and Pullen, R. L.: Arch. Int. Med. **73**:365,1944.
3. Jamison, C., and Hopkins, J.: New Orleans M. & S. J. **93**:580,1941.
4. Castleden, L. I. M., and Hamilton-Paterson, J. L.: Brit. M. J. **2**:478,1942.
5. LeMone, D. V.; Scott, W. G.; Moore, S. and Koven, A. L.: Read at the Thirty-Second Annual Meeting of the Radiological Society of North America, December 1946.

Attempts to reproduce the disease in animals were reported as unsuccessful.[2]

The present investigation was undertaken to determine experimentally the pathogenic properties of bagasse fibers and of the microbial contents of representative suspensions. The possibility that other factors, such as hypersensitivity, play a role in the development of the experimental disease was also explored.

MATERIAL AND METHODS

Five lots of bagasse were obtained by courtesy of Dr. W. H. Reinhart, of the Louisiana State Department of Health. These were arbitrarily called A, B, C, D and E. Lots A and E were employed for these experiments. Lot A had been baled in January 1944 and stacked for two years. Lot E was a sample of dust collected from rafters near the conveyer in a bailing shed.

For experimental intravenous injections, bagasse was ground in a Waring blender, sterile saline solution added and the suspension filtered through sterile cheesecloth. The suspended particles varied in size from less than 1 micron to 15 microns. They were counted in a hemocytometer, and the suspension was adjusted so that 7,500 particles were present in a cubic millimeter. For intratracheal application, suspensions containing 30,000 to 35,000 particles per cubic millimeter of a size up to 100 microns were made up.

Rabbits and guinea pigs of similar weights and ages were employed in the experiments.

RESULTS

Intravenous Administration.—Rabbits receiving intravenous injections of a suspension of bagasse A (5 doses of 5 cc. each at three day intervals) rapidly became sick, listless and dyspneic and showed paralysis of the hindlegs. One of them died on the third day, and the three remaining animals were so sick that they were killed on the fourth day. When, however, an aliquot of the bagasse suspension was autoclaved (twenty minutes at 15 pounds [6.8 kg.] of pressure) similar amounts could be given without any apparent ill effect. For control purposes, 2 animals given the autoclaved suspension were killed at an interval corresponding to that of deaths in the first group. The lungs and parenchymatous organs of the animals given the fresh bagasse suspension showed extensive foci of necrosis and marked cellular reaction (fig. 1), indicating that this suspension contained a powerful pathogenic agent. The presence of mycelia and spores (fig. 2) in the organs showing the severest damage served as a clue to the nature of the agent. It is noteworthy that similar lesions could be produced by a single injection of a more concentrated suspension.

In contrast, preparations representing the rabbits given injections of the autoclaved bagasse suspension revealed only small granulomatous lesions of the foreign body type in the lung (fig. 3). Necrosis was absent, and the other organs were free of lesions.

It was then established that autoclaved bagasse suspensions could be given repeatedly without any adverse effect. A rabbit that received 16 intravenous injections, totaling 74.5 cc., over a period of thirty-nine days was killed thirty-eight days after the last injection. Lesions were not seen grossly or microscopically except for an occasional small group of monocytes containing small, irregularly shaped foreign bodies, noted in sections of the lung.

These results suggested that one or several types of micro-organisms present in the bagasse were the pathogens, while the fiber itself under the conditions tested produced only a foreign body reaction of no apparent consequence to the experimental animal.

In the bacteriologic studies five different micro-organisms were isolated from bagasse A suspension and cultured on various mediums under aerobic conditions: Organism 1 gave the reactions of the group of organisms designated bacteriologically as Bacillus polymyxa. Organism 2 was a characteristic form of Aspergillus fumigatus. Organism 3 was an aerobic member of the Actinomyces group. Organism 4, while differing in gross appearance from 2, showed microscopic characteristics that place it in the Aspergillus fumigatus group also. Organism 5 was an aerobic motile spore-formig gram-positive rod which failed to liquefy gelatin and had many, though not all, of the characteristics of the Bacillus circulans group.

Similar results were obtained with the other lots of bagasse except that organism 4 could not be isolated from lot B.

Suspensions were prepared by washing the agar slants with saline solution. They were standardized bacteriologically to contain approximately 10,000 organisms per cubic millimeter. The elements of the Aspergillus and Actinomyces cultures were similarly counted by attempting, as far as possible, to estimate the number of particles—mycelia or spores—present. It was planned to inject 5 cc. of one or another of the suspension intravenously into each rabbit twice. Suspensions of 1, 3, 4 and 5 were well tolerated. Rabbits, however, which had received a single injection (5 cc.) of suspension 2 (aspergillus) invariably died or were so sick two to three days after the first injection that it was preferred to put them to death.

The lungs, hearts, livers and kidneys of these rabbits showed numerous lesions of a necrotizing character. Mycelia were sometimes histologically demonstrable, and A. fumigatus was recovered from a blood culture at autopsy.

Sections representing the rabbits inoculated with the other micro-organisms and killed at similar intervals revealed that organisms 1 and 3 produced only minimal foreign body reaction in the lung. The cellular reaction of the lungs of the animals inoculated with organisms 4 and 5 was also slight, but abscess formation was observed in the liver in both instances. Numerous multinucleated giant cells were noted within the granulation tissue. Structures phagocytosed in their cytoplasm suggested that the giant cells were macrophages which had ingested some of the micro-organisms.

Three rabbits that were given suspensions of bagasse E in amounts and over periods similar to those employed in the first two groups withstood the treatment well. Two of them, on histologic study, showed minimal pulmonary lesions, one of them also a renal abscess, while the lungs of the third animal showed numerous foci of mononuclear exudate and of giant cell formation; polymorphonuclear leukocytes were rare, and necrosis was absent.

Intratracheal Administration.—The fact that bagasse injected intravenously produced severe lesions did not necessarily imply that it would call forth a similar reaction when introduced by way of the respiratory tract. A group of rabbits received, therefore, bagasse suspensions by intratracheal insufflation. For this the animals were under combined local and light ether anesthesia.

Each rabbit was given 10 cc. of suspension, an amount of fluid that is absorbed from the lungs within seventy-two hours.[6] For control purposes aliquots of the

6. Courtice, F. C., and Phipps, P. J.: J. Physiol. **105**:186, 1946.

suspensions were either autoclaved or formaldehydized by addition of 10 per cent (volume) of a 40 per cent solution of formaldehyde. After three to four days the formaldehyde solution was removed by centrifuging the particles and resuspending them in saline solution. These suspensions were sterile on culture. The animals were killed after ten days. Microscopic sections of the lungs of rabbits treated with the suspension of fresh bagasse showed numerous large pneumonic foci (fig 4). The exudate was composed of polymorphonuclear leukocytes, monocytes and multinucleated giant cells, with frequent disintegration of the exudate cells. The giant cells were of the foreign body type and contained variously shaped cytoplasmic defects which under polarized light sometimes corresponded to double-refractile, apparently unstained foreign bodies. The interstitium was infiltrated by round cells. There was fibroblastic proliferation at the periphery of the lesion. Some bronchial lumens contained desquamated epithelial and disintegrated exudate cells. An occasional vessel was occluded by granulation tissue and its wall infiltrated by small round cells (fig. 5). One liver revealed numerous small foci of necrosis and infiltrating polymorphonuclear leukocytes.

In sections of the lungs of the control animals, lesions were infrequent, small and of granulomatous character. Giant cells were fairly numerous, but polymorphonuclear leukocytes were encountered only occasionally, and cellular disintegration was absent (fig. 6). Fibroblastic proliferation was more intense than in the first group. The vessels were free of lesions.

Suspensions of bagasse E were administered to 2 rabbits. After ten days their lungs contained fairly frequent foci of pneumonic exudate, with monocytes being prevalent in the larger and polymorphonuclear leukocytes in the smaller lesions. Multinucleated giant cells were also present. Cellular disintegration and interstitial infiltration were, however, less prominent than in the group which had received bagasse A.

Guinea pigs were subjected to similar procedures, with suspension of bagasse E, except that the amount insufflated was 3 cc. Sections of the lungs of 2 animals obtained at a ten day interval revealed several foci of a polymorphonuclear and monocytic alveolar exudate. Several alveoli in these groups contained multinucleated giant cells, one to each. Sporelike foreign bodies were seen in the cytoplasm of these cells. There was no necrosis, and no changes were found in the other organs.

Intracutaneous Tests.—It has been stated earlier that intracutaneous tests carried out with extracts of bagasse by two groups of authors yielded different results. This problem was therefore also investigated. Rabbits which had received two intravenous injections each of 5 cc. of bagasse suspension over a period of six days were tested by intracutaneous injection of 0.05 cc. of Seitz filtrates prepared from saline suspensions of cultures of each of the five micro-organisms mentioned earlier. The tests were made thirteen days after the first injection of the bagasse suspension.

With filtrates 1 and 4 there was a reddening of the skin within a few hours after the injection, and the reaction increased in intensity until about twenty-four hours later. A slight reaction was observed in one instance with filtrate 2. The other filtrates and saline solution, which was used as control, gave negative results. A normal rabbit, however, tested with identical preparations showed similar results.

That sensitization is not an essential factor in the genesis of the experimental disease was also indicated by the fact that a single injection of a more concentrated suspension of bagasse A produced lesions similar to those due to repeated injections.

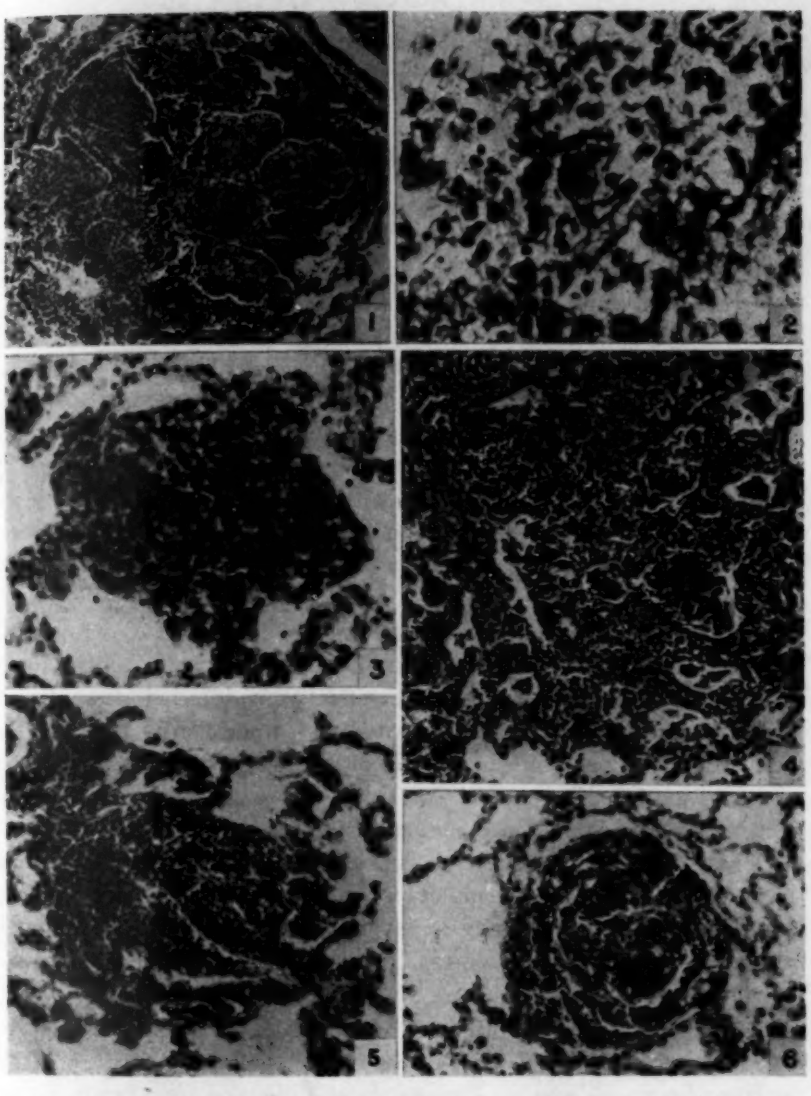

Fig. 1.—Rabbit given intravenous injections of a suspension of unheated bagasse A; third day; lung; focus of necrotizing pneumonia; Masson stain; × 75.

Fig. 2.—Rabbit given intravenous injections of a suspension of unheated bagasse A; third day; kidney; mycelial fragment and spore; Masson stain; × 476.

Fig. 3.—Rabbit given intravenous injections of a suspension of autoclaved bagasse A; third day; lung; granulomatous lesion; hematoxylin and eosin; × 238.

Fig. 4.—Rabbit given intratracheal insufflation of fresh bagasse; tenth day; lung; pneumonic exudate of polymorphonuclear leukocytes, monocytes and foreign body giant cells; hematoxylin and eosin; × 136.

Fig. 5.—Rabbit given intratracheal insufflation of fresh bagasse; tenth day; lung; vessel occluded by granulation tissue: hematoxylin and eosin × 156.5.

Fig. 6.—Rabbit given intratracheal insufflation of autoclaved bagasse; tenth day; lung; foreign body granuloma; hematoxylin and eosin; × 177.

COMMENT

In the cases reported so far human bagasse disease occurred after an exposure of several weeks or months. The pathologic changes described here resulted from short term experiments and should not be taken, therefore, as a basis for comparing the lesions morphologically.

Suspensions of unheated bagasse given intravenously proved fatal to rabbits except when given in small doses. The fact that numerous necrotizing lesions with fragments of fungi demonstrable within them were found in the kidney and the liver while the highly refractory bagasse fibers were not seen there, together with the fact that there were no lesions in these organs in animals given injections of autoclaved bagasse, suggests that the fiber itself does not contribute to the causation of the experimental disease. It may be assumed, however, that the process of autoclaving changed the structure of the fiber, rendering it less harmful. Thus, resort was taken to formaldehydizing, a procedure that would affect the micro-organisms of the suspension and change only amino groups of protein particles possibly present in the bagasse fibers. The pathogenicity of bagasse, however, was equally abolished by the second procedure.

Among the micro-organisms isolated from bagasse, the aspergilli proved to be the most harmful for rabbits. These are ubiquitous fungi and are known not only for their toxic effect when injected intravenously into animals but also for their occasional pathogenicity for man. In view of the cosmopolitan distribution of A. fumigatus in the soil and in vegetable matter, it is difficult to attribute human bagasse disease to these organisms alone, since similar respiratory disease has not as yet been noted among many other occupational groups equally exposed to these fungi. Experiments employing prolonged exposure or providing longer intervals are necessary to decide whether aspergillosis, alone or in combination with other factors, simulates in its morphologic aspects human bagasse disease.

A slight but distinct difference was noted between the lesion produced by bagasse A and that by bagasse E, the changes produced by the latter being less severe. Bagasse disease has, however, rarely if ever occurred in men working in places where bagasse E was collected, and thus its pathogenicity for rabbits would represent a failure to supply the experimental corollary. Among the microbial contents of bagasse E, however, were also aspergilli, and it may well be that the particular susceptibility to these fungi enhanced the severity of the lesions in rabbits. The less serious character of the lesions occurring in guinea pigs would support this assumption.

SUMMARY

Final interpretation of the results of these experiments has to be postponed until more information concerning the pathogenicity of the micro-organisms and of the fibers described will be available. Preliminarily it may be said, however, that the bagasse fiber itself under the test conditions described is a rather inert material, that the acute inflammatory process caused by bagasse is due to micro-organisms, probably fungi, attached to the bagasse, and that the lesions in no instance resembled those in human or experimental silicosis.

RHABDOMYOSARCOMA OF THE BLADDER

Report of a Case and Review of the Literature

HERMAN L. KRETSCHMER, M.D.
CHICAGO

NONEPITHELIAL tumors of the urinary bladder are of infrequent occurrence, and sarcoma is the most rare. The rarity of sarcoma of the bladder is evidenced by its low incidence in reported collections of tumors of the bladder. Albarran [1] observed 2 sarcomas among 89 vesical neoplasms; Caulk,[2] 1 in 303; Davis,[3] 2 in 41; Egger,[4] 1 in 83; von Frisch,[5] 1 in 300; Scholl,[6] 1 in 262; Ratliff and Valk,[7] 3 in 552; Wheelcock [8] encountered 3 in 58,437 surgical specimens and 2,783 autopsies at the Deaconess Hospital, Boston, and at Pondville Hospital, Wrentham, Mass., only 1 sarcoma was found among 288 primary vesical tumors; Pack and LeFevre [9] in a survey of neoplastic diseases at the Memorial Hospital for the Treatment of Cancer and Allied Diseases, New York, from 1917 to 1929 found only 2 cases of sarcoma of the bladder among 19,129 cases of tumor. Crane and Tremblay [10] reviewed the literature in 1943 and were able to collect only 151 cases of primary vesical sarcoma.

Many histologic types have been reported: angiosarcoma, leiomyosarcoma, fibromyxosarcoma, neurogenic sarcoma, fibrosarcoma, rhabdomyosarcoma, rhabdomyomyxosarcoma and osteogenic sarcoma. No detailed discussion of sarcoma in general will be undertaken, as several excellent reviews have appeared in the literature. Apart from chondrosarcoma and osteogenic sarcoma, rhabdomyosarcoma and rhabdomyomyxosarcoma are among the rarest tumors to be found in the bladder.

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1. Albarran, J.: Ann. d. mal. d. org. génito-urin. **15**:785, 1897.
2. Caulk, J. R.: J. Urol. **16**:211, 1926.
3. Davis, L.: Ann. Surg. **43**:566, 1906.
4. Egger, O.: Ztschr. f. urol. Chir. **6**:175, 1921.
5. von Frisch, A.: Wien. klin. Wchnschr. **20**:1205, 1907.
6. Scholl, A. V.: Surg., Gynec. & Obst. **34**:189, 1922.
7. Ratliff, R. K., and Valk, W. L.: J. Urol. **42**:559, 1939.
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To date there have been reported 16 cases of rhabdomyosarcoma and rhabdomyomyxosarcoma, and my own case is the seventeenth to be recorded. Only 15 cases of those recorded in the literature have been summarized in the table, as the article by Janu and Stolz [11] was not available.

REPORT OF CASE

Mr. G. J. H., aged 53, entered the Presbyterian Hospital, Chicago, July 13, 1942. He was married and had two children, living and well. At the age of 35 he suffered an attack of gonorrhea, from which he made a complete recovery. The family history was noncontributory. Two weeks prior to hospitalization profuse hematuria developed. The day following the onset of the hematuria frequency of urination developed and continued both day and night. Some burning and urgency of urination were noted.

Examination showed head, neck, heart, lungs and abdomen normal. There was a small hydrocele on the the right side. .Rectal examination revealed no abnormality. The right lower extremity showed marked varicose veins. The Kahn test was negative. Roentgen films, including intravenous pyelograms, showed no evidence of disease. The blood count showed 12,300 leukocytes per cubic millimeter; otherwise the blood was normal. The urinalysis showed albumin (1 plus); the urine contained no sugar, and the sediment showed red blood cells. All cultures of the urine were sterile except the ascitic fluid broth, which showed a scant growth of small gram-negative cocci after four days.

Cystoscopic examination showed a tumor, the size of a large walnut, in the dome of the bladder.

Suprapubic cystotomy was done on July 21, with resection of the tumor, including the involved wall of the bladder and fulguration of the area of excision. The patient made an uneventful recovery and was discharged August 5.

He applied for insurance in May 1946. The report from the insurance company, dated May 29, 1946, stated that physical examination gave negative results, that the urinalysis revealed no abnormality and that the patient had no symptoms.

On September 26, cystoscopic examination showed no abnormality, and there was no sign of tumor.

The pathologic diagnosis, made by Dr. George M. Hass, of the Presbyterian Hospital, was: rhabdomyosarcoma of the urinary bladder. His report follows:

"There are numerous mitotic figures, and the cells are poorly differentiated. It is difficult to classify the tumor, which is not the usual type of fibrosarcoma. The cells are in keeping structurally with cells of leiomyosarcoma or those of rhabdomyosarcoma. This means that the tumor has cells in it that are very similar to those that are found in rhabdomyosarcoma of the heart, the skeletal muscle or mixed tumors, especially those of the kidney. Inasmuch as tumors of this type, especially mixed tumors, have been frequently found in relation to the urinary tract and occasionally in relation to the bladder, I am inclined to classify this tumor as rhabdomyosarcoma."

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11. Janu, M., and Stolz, J.: Časop. lék. česk, **71**:15, 1932. (Original article not available for perusal.)

Recorded Cases of Rhabdomyoma and Rhabdomyomyxosarcoma

Author	Sex	Age	History	Result	Location of Tumor	Type of Tumor
Mönckeberg, J. G.: Virchows Arch. f. path. Anat. **187**: 471, 1907	F	23	Hematuria. Removal of tumor. Recurrence. Second removal	Death	Posterior wall of bladder	Rhabdomyosarcoma
MacKenzie, D. W., and Chase, W. H.: J. Urol. **19**: 315, 1928	F	69	Hematuria; pain in left loin, radiating to bladder; nausea and vomiting; loss of weight and strength. Operation for nephritis. Readmission for recurrence of symptoms. Cystoscopy with diagnosis of cancer of bladder. Suprapubic cystotomy	Death	Floor of bladder and entire trigone	Rhabdomyosarcoma of bladder extending directly into left ureter, with metastases in duodenum, portal lymph nodes and liver
Houette [13]	M	13 mo.	Urinary difficulties since birth; mass in pelvic region; uremia	Death	In a congenital diverticulum of bladder	Rhabdomyosarcoma
White, H. P. W.: Proc. Roy. Soc. Med. **22**: 1382, 1929	Not stated	1 yr. 10 mo.	Swelling in lower part of abdomen; urine turbid with pus. Cystoscopy and cystogram led to diagnosis of tumor of bladder. Excision of tumor with partial resection of bladder	Result not stated, but prognosis poor	Anterior wall of bladder	Rhabdomyosarcoma
Monserrat, J. L., and Garcia, A. E.: Hosp. argent. **3**: 856, 1933	M	43	As original article was not available for perusal, data on history cannot be supplied; data given here have been obtained from other authors	Death	Right wall of bladder	Rhabdomyosarcoma
Welfield, J.; Hill, L., and Hillebrand, J. G.: J. Urol. **36**: 150, 1936	F	Infant	Incontinence, abdominal pain, hematuria. Cystogram. Diagnosis: vesical tumors. Cystotomy. Microscopic examination made of some pieces of tumor. No treatment	Death	Not stated	Rhabdomyomyxosarcoma
Idem	M	2	Straining on urination, frequency; later, incontinence; paroxysmal pains. Exploratory cystostomy. Removal of tumor of bladder. Third day after operation tumor tissue noticed fungating through bladder opening. Roentgen irradiation, application of radium and excision of mass	Death	Anterior wall of bladder	Rhabdomyomyxosarcoma
Vermooten, V.: J. Urol. **42**: 126, 1939	F	20 mo.	For 6 months difficulty in passing urine; blood in urine; constipation. Catheterization for retention of urine	Death	Trigone	Rhabdomyosarcoma
Ratliff and Valk [7]	M	6	Nocturia, frequency, straining and dysuria for 6 mo. terminating in acute retention 3 days prior to admission. Suprapubic inspection showed neoplastic prostate and complete infiltration of vesical neck. Tumor stripped from base, and base cauterized. Radium used 25th day following removal of recurrent tumor; 5 mo. later, tumor completely filled bladder; enucleation; 2 mo. later, excision; 1 mo. later, recurrence	Death	Entire bladder	Rhabdomyomyxosarcoma
Idem	M	65	Progressive intermittent hematuria for 8 mo.; frequency, straining and mild tenderness over bladder area. No treatment	Death	Tumor attached to pedicle 2 cm. above and to left of left ureteral orifice	Rhabdomyosarcoma

Idem	M	65	Progressive intermittent hematuria for 8 mo.; frequency, straining and mild tenderness over bladder area. No treatment	Death	Tumor attached to pedicle 2 cm. above and to left of left ureteral orifice	Rhabdomyosarcoma
Uhlmann, E.; Grossman, A., and Calvin, J. K.: Ann. Surg. 109:624, 1939	M	10 mo.	Pain on urination. Suprapubic cystotomy. 1 week later fungating mass had grown out to anterior abdominal wall at site of cystotomy. Radiation therapy	Death	Tumor arose from outer coats of wall of bladder; the space of Retzius completely filled by tumor; mucosa apparently not involved	Rhabdomyosarcoma
Hunt [13]	M	Newborn infant	72 hr. after birth cyanosis of legs and abdominal distention; painful mass in suprapubic area. Catheterization for acute retention; development of urinary fistula. 65 days after birth, cystostomy; nodular mass covering floor of bladder with greater concentration around neck of bladder; biopsy showed rhabdomyoma; 2d biopsy, rhabdomyosarcoma. 179 days after birth, total cystectomy, prostatectomy, transplantation of ureters. Later, transverse colostomy and right ureterosigmoid anastomosis	Death	Floor of bladder; greater concentration around bladder neck	Rhabdomyosarcoma
Idem	M	2½	Pain on urination and retention of urine. Cystoscopy showed tumor of bladder. Suprapubic cystostomy with fulguration of tumor; roentgen therapy. Rapid recurrence of tumor; hydronephrosis. Right nephrostomy for drainage, transfusions, total cystectomy and prostatectomy, with transplantation of left ureter. Several subsequent recurrences; mass eventually closed off ureter	Death	Entire bladder	Rhabdomyosarcoma
Khoury and Speer [14]	M	Infant	2 mo. after birth, cystotomy for umbilical fistula; nodular mass found in bladder; later, diarrhea, vomiting, pyrexia and voiding of urine through suprapubic wound; these symptoms lasted 11 mo. Total cystectomy and abdominal ureterostomy followed by 2 stage bilateral ureterosigmoidostomy. Growth had caused hydronephrosis, ascending urinary infection and urachal fistula	Death	Trigone; growth surrounded the urethra and infiltrated wall of bladder, prostate and verumontanum	Rhabdomyosarcoma
Hirsch and Gasser [15]	M	5	Frequency, urgency, voiding small quantities of urine; distended bladder; rectal tumor. Suprapubic cystotomy; tissues removed for histologic examination. Suprapubic cystotomy. Roentgen therapy	Death	Extramural, in the tissues behind trigone and prostatic urethra	Rhabdomyosarcoma
Kretschmer, H. L.: Arch. Path., this issue	M	53	Hematuria, frequency, burning and urgency of urination. Cystoscopic examination: vesical tumor. Suprapubic cystotomy; resection of tumor, including wall of bladder; fulguration of area of excision	Recovery. Cystoscopic ex. 4 yr. 2 mo. after operation showed bladder normal	Dome of bladder	Rhabdomyosarcoma

A specimen was sent to the Army Medical Museum, and Dr. Raymond O. Dart, who at present is in charge of the Tumor Registry of the American Urological Associations, reported as follows:

" . . . it has been found expedient to use the generic term 'myosarcoma' in many cases. The reason for this is that the cellular composition of many of the prostatic and vesical tumors of muscle varies from undifferentiated elements all the way to frankly striated cells, often varying during the progression of the disease. The present tumor contains some large acidophilic elements, and it is highly probable that it is rhabdomyosarcoma."

COMMENT

Rhabdomyosarcoma is the cancerous variant of rhabdomyoma, and both types have their origin in striated muscle fibers. Rhabdomyoma and rhabdomyosarcoma have been reported as occurring in the heart, the skeletal musculature, the testicles, and the vagina, but the bladder has seldom been the seat of either tumor. However, one can only speculate as to the embryogenesis of these rare tumors, and there is no unanimity of opinion.

Wilms [11a] and Houette [12] expressed the belief that each of these tumors arises from embryonal heterotopia due to an anomaly of the development of the wolffian canal. Shattock was inclined to think that rhabdomyoma arises from vagrant sarcoblasts which have abnormally extended or have been displaced beyond their usual deeper limits into the subepithelial tissue of the bladder. Hunt [13] stated that although one can never prove the origin of these tumors, it is probable that they arise from the striated muscle elements normally present in the anterior part of the prostate and in the region of the internal sphincter and adjacent area of the trigone. Khoury and Speer [14] also expressed the opinion that it is plausible that the neoplasm containing striated tissue could arise from the striated muscle elements normally present in the sphincter of Henle, but this theory does not explain how these tumors can arise at a distance from the neck of the bladder. They also called attention to the fact that rhabdomyosarcoma of the prostate may be confused with rhabdomyosarcoma of the bladder.

Hirsch and Gasser [15] reported an extramural rhabdomyosarcoma of the urinary bladder that occurred in the tissue behind the trigone and the prostatic urethra and mentioned that extramural rhabdomyosarcoma has been reported as primary in the prostate; they are inclined

11a. Wilms, M.: Die Mischgeschwülste, Leipzig, Arthur George, 1899.

12. Houette, C.: Ann. d'anat. path. **6**:267, 1929.

13. Hunt, R. W.: New York State J. Med. **43**:513, 1943.

14. Khoury, E. N., and Speer, F. D.: J. Urol. **51**:505, 1944.

15. Hirsch, E. F., and Gasser, G. W.: J. Urol. **51**:517, 1944.

to favor the conclusion that these tumors are primary in the prostate or that they may belong with rhabdomyosarcoma occurring along the vas deferens. They stated,

> . . . The hypoplasia (or atrophy) of the right vas deferens observed with the rhabdomyosarcoma of this report suggests that there have been disturbances in growth or development of tissue about the spermatic cord in the region of the prostate. The rhabdomyosarcoma cell invasion of the puerile prostate and seminal vesicle tissues, which is comparable to the cell invasion of the wall of the urinary bladder, leaves the impression also that both the prostate and the urinary bladder have been invaded secondarily.

Since there is confusion as to the origin of rhabdomyosarcoma of the bladder and it has been suggested that it may start from the prostate, I wish to call attention to the fact that the tumor in my case was located in the dome of the bladder. This excludes the possibility that it took its origin in the prostate, and excludes also the possibility that it took its origin in the vas deferens or the seminal vesicles, invading the bladder secondarily, as has been stated by several of the previously mentioned authors.

PROGNOSIS

An analysis of the cases reported shows that the prognosis is poor in patients having this highly malignant and rapidly recurring tumor. All of the cases resulted fatally. In the case reported here there has been no recurrence to date and the patient is free of symptoms four years and two months after operation.

SUMMARY

A review of the literature and my own case brings the number of reported cases of rhabdomyosarcoma of the urinary bladder to 16. Eleven of the patients were under 10 years of age.

LIMITATION OF SPREADING FACTOR IN INJURED RATS

ISOLDE T. ZECKWER, M.D.
PHILADELPHIA

IT IS NOW well established that after many injuries of the body the adrenal cortex, by a reaction mediated through the anterior lobe of the pituitary gland, promptly discharges a hormone which destroys lymphocytes, thereby releasing antibodies and causing shrinkage of lymphatic tissue. This constitutes a coordination of defense reactions when the injury is a bacterial agent. However, this chain of reactions occurs, ending in destruction of lymphocytes, whether the injurious agent is a bacterial or whether it is a physical or a chemical one. Presumably, the lymphocyte when disrupted may release other protective substances besides antibodies.

Since under many pathologic conditions in which bacteria are not present, such as healing of bland infarcts, degeneration of goiters and healing of sterile wounds, lymphocytes occur in tissue simultaneously with fibroblasts, it has been thought that the lymphocytes might make possible the laying down of collagen by connective tissue cells. In bacterial infections, it is now well known, the spread of the inflammatory process is greatly enhanced by hyaluronidase, which hydrolyzes the hyaluronic acid in the intercellular substance formed by connective tissue. If in sterile areas of destruction of tissue hyaluronidase should be present, it would seem possible that the healing might be aided by lymphocytes releasing a substance that limits the action of hyaluronidase and thus creates the conditions which permit connective tissue cells to lay down collagen. If this were so, it would give new meaning to the adrenocortical-lymphatic tissue reaction to injury.

Certain observations reported in the literature suggest that the spread of hyaluronidase may be influenced by pituitary-adrenocortical activity. It has been shown by Weinstein [1] that an anterior pituitary extract when injected repeatedly into rabbits caused limitation of the spread of testicular extract injected intracutaneously. Cahen and Granier [2] observed that spread of hyaluronidase is restricted in animals given previous injections of morphine. Epinephrine, also, caused restriction of the spreading fac-

From the Department of Pathology, University of Pennsylvania School of Medicine.

1. Weinstein, L.: Yale J. Biol. & Med. **12**:549, 1940.
2. Cahen, R. L., and Granier, M.: Yale J. Biol. & Med. **16**:257, 1944.

tor, according to investigators cited by Cahen and Granier.[2] Both morphine and epinephrine were listed by Selye [3] as damaging agents which incite the "alarm reaction" resulting in shrinkage of lymphatic tissue which is now known to be caused by pituitary-adrenocortical activity. Morphine is known to stimulate the adrenal medulla to secrete epinephrine, and epinephrine has been shown by Vogt [4] and by Long and Fry [5] to cause secretion on the part of the adrenal cortex.

In view of these data and in view of the important part played by hyaluronidase in enabling bacteria to spread through connective tissue, it was thought of interest to see whether limitation of spread occurred during the pituitary-adrenocortical-lymphatic tissue reaction which occurs as a result of various injuries of the body (bacterial, chemical or physical).

With this idea in mind, it was determined to study quantitatively the spread of ink combined with a spreading factor in the skin of the abdomen of rats subjected to injury of the leg muscles and to compare this with the spread in uninjured rats. The dose and intervals of time used were those which had proved effective in activating the pituitary-adrenocortical dissolution of lymphocytes in previous experiments.[6]

EXPERIMENTAL PROCEDURE

Fourteen pairs of rats were used, each pair of the same sex and either litter mates or of the same weight. One of each pair received intramuscularly 0.5 cc. of solution of formaldehyde U. S. P. diluted 1:25 in each of the four legs, one at a time, over a period of two days. In a few rats trypsin solution was injected instead of solution of formaldehyde, to cause injury. The control either received no injection or received injections of isotonic solution of sodium chloride. At intervals varying from two to nineteen hours after the last injection, the rats were anesthetized with ether, the hair was removed from the chest and the abdomen by electric clippers, and intracutaneous tests were made for spread. The spreading factor used was, as a matter of convenience, derived from rat testes rather than from bacteria. The testes were ground first in water; then an equal volume of 1.8 per cent sodium chloride solution was added, and the mixture was centrifuged. As many cubic centimeters of solution were added as there were grams of tissue. This preparation was kept in the refrigerator, during which time it increased in potency; it was subsequently diluted with saline solution for a convenient degree of spread. The supernatant liquid was drawn up to 0.15 cc. in a tuberculin syringe, then 0.05 cc. of india ink (not waterproof) suspension which had been diluted previously 1:2 with 0.9 per cent salt solution was drawn into the syringe, and the mixture was injected into the skin of the abdomen. A control injection of 0.15 cc. of a 0.9 per cent sodium chloride solution with 0.05 cc. ink solution was made in the skin of the thorax. When the formaldehyde solution had been injected into the rat, a great

3. Selye, H.: Endocrinology **21**:165, 1937.

4. Vogt, M.: J. Physiol. **103**:317, 1944.

5. Long, C. N. H., and Fry, E. G.: Proc. Soc. Exper. Biol. & Med. **59**:67, 1945.

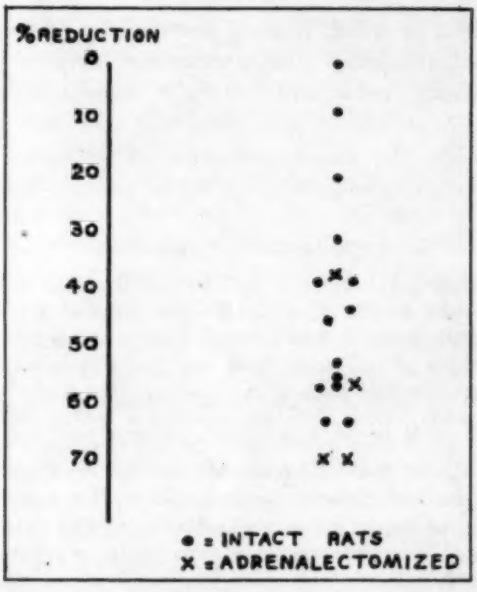
6. Zeckwer, I. T.: Proc. Soc. Exper. Biol. & Med. **60**:66, 1943

deal of edema occurred at the site and sometimes extended toward the abdomen. The ink was injected as far as possible from the edematous area.

The spread of the dye was traced on cellophane at appropriate intervals. One and two hours after the injection of ink were found to be convenient times for reading the spread. The area was measured by a planimeter in square inches

Experimental Observations

Experimental Group	Mean Spread of Ink		Mean Percentage Reduction of Spread in Injured Rats as Compared with Controls
	In Control Rats	In Injured Rats	
14 pairs of intact rats.....	2.01 sq. in. (13 sq. cm.)	1.12 sq. in. (7 sq. cm.)	44%
4 pairs of adrenalectomized rats..	2.60 sq. in. (16.5 sq. cm.)	1.16 sq. in. (7.5 sq. cm.)	56%



Percentage reduction of the spread of a mixture of ink and testicular extract in injured rats as compared with uninjured rats.

(table), and the amount of spread observed in the injured rat was calculated as a percentage change of that observed in the control rat of each pair (figure). As different concentrations of hyaluronidase were used as spreading factors from day to day, comparisons cannot be made between all of the injured rats and all of the controls. On the contrary, each test rat should be compared with its own control which received the same spreading factor.

It was soon obvious that injured rats showed marked limitation of the spread. It then became necessary to see whether this depended on adrenocortical activity. The experiments were repeated with four pairs of adrenalectomized rats. Adrenalectomy was performed through a single midline incision of skin carried down the back, with incision through muscles and peritoneum on each side of the vertebral

column, so as to avoid any traumatizing of the abdominal area. The rats were maintained in good condition on saline drinking solution for one to three weeks before the spread was determined. The tests for spread were made two to five hours after the last injection of formaldehyde solution. The adequacy of the removal of functioning adrenocortical tissue was evidenced by (1) the fact that some animals died during the day following the last injection of formaldehyde solution, or (2) the fact that there was hypertrophy of lymphatic tissue with failure to shrink after injections of formaldehyde solution in those rats which were killed after completion of the experiment, or (3) the fact that spontaneous death of the rat occurred when salt was withdrawn from the drinking water. As will be seen from the chart, the injured adrenalectomized rats showed just as much limitation of the spread of ink as did the intact injured rats. Hence, responsibility of the adrenocortical-lymphatic tissue reaction can be ruled out.

COMMENT

Since the restriction of spread does not depend on adrenocortical activity, the only other obvious possibility is that the edema of the legs following injury might lead to relative dehydration of the abdominal area given injections of dye and that this dehydration might limit the spread through connective tissue. Duran-Reynals [7] stated that when testicular extract is injected directly into an edematous area the spread is restricted. No data were found on the effect of dehydration. According to Duran-Reynals [7]: "It would seem that the spreading is a phenomenon in which vasomotor or other nervous reactions play no part." When the spreading factor is injected directly into an area of injury or inflammation, the spread is limited, but in the present experiments the site of injection of the spreading factor was distant from the injured area. Hechter [8] found that ischemia from exsanguination did not influence the spread of hyaluronidase and concluded that the spread "is not influenced by changes in skin blood flow."

SUMMARY

The spread of a mixture of ink and testicular extract (hyaluronidase) injected intracutaneously into the abdomen was restricted in rats whose leg muscles had been injured by injections of formaldehyde solution. This restriction also occurred in adrenalectomized rats, and consequently it did not depend on the adrenocortical-lymphatic tissue reaction to injury.

7. Duran-Reynals, F.: Bact. Rev. **6**:197, 1942.
8. Hechter, O.: J. Exper. Med. **85**:77, 1947.

HISTOLOGIC ASPECTS OF THE SKIN IN THE LATE STAGES OF TRENCH FOOT

MATTHEW BLOCK, M.D.*
CHICAGO

THE PURPOSE of this investigation was to study the pathologic aspects of the chronic form of trench foot, to determine the mechanisms responsible for the accompanying clinical symptoms and thus to lay the groundwork for a more rational therapy. Although from the point of view of pathology a great deal of information about trench foot based on amputation material has been accumulated,[1] there has been little concerning the late sequelae, especially in cases in which there was no loss of tissue. This investigation was based on biopsy specimens taken from 15 patients with uncomplicated trench foot in the chronic state and from 8 controls.

Trench foot occurred in these patients as a result of their being exposed to cold wet weather over periods varying from days to weeks. Other factors resulting from unavoidable circumstances of combat adversely affected the course of the disease. Some of the men were unable to change their wet shoes or socks for as long as a week or two; others were forced to live in the snow without shelter for long periods. Because of the tactical situation, many were kept on their feet during the early stages of the disease, others were forced to lie in cramped positions in foxholes, in melted snow up to their waists for hours and days at a time.

In its most characteristic and severe form, trench foot occurred primarily in combat infantrymen. More specifically, it was predominantly a disease of the lower ranks of the ground combat troops, who bore the brunt of the fighting under conditions conducive to the development of the disease.

Trench foot closely resembles other neurovascular diseases of the extremities caused by exposure to cold, as immersion foot,[2] high altitude

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From Camp Butner General Hospital.

The photographs were taken by Mr. Roy Reeve and his staff, of the Army Institute of Pathology.

*Formerly Captain, Medical Corps, Army of the United States; now in the Department of Medicine, University of Chicago.

frostbite,[3] pernio [4] and frostbite.[5] There is still a great deal of discussion concerning the interrelationships and possible fundamental identity of all these processes. However, trench foot may be separated from the others on the basis of the history that the patient had been exposed to cold wet weather under the circumstances described.

Although trench foot is as old as war in cold wet weather, the first accurate clinical account of the acute phase was written by Larrey [6] on the basis of his experience during Napoleon's retreat from Moscow. He described the pale, swollen appearance of the feet, coincident with numbness and paresthesias, and the supervening painful erythematous stage, followed by dry gangrene. In the course of several weeks he observed exfoliation of the mumified gangrenous tissue or, in the more severely injured, demarcation and slough of the mummified tissue. The details of this acute phase of trench foot and related diseases may be found in many recent articles.[7]

CLINICAL SYMPTOMS AT TIME OF BIOPSIES

The biopsies were performed on patients from three to four months after evacuation. In the more severe lesions studied, a demarcation zone had formed, and the gangrenous tissues had shrunk so that spontaneous amputation was obviously imminent. In the less severe lesions the shell of superficial gangrenous tissue had been exfoliated and new skin had already developed to cover the toes. All but 2 men were ambulant, although an attempt was made to keep those whose lesions were more severe in bed, to prevent secondary infection.

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1. (*a*) Friedman, N. B.: Am. J. Path. **21**:387, 1945. (*b*) Siegmund, H.: Zentralbl. f. Chir. **70**:1558, 1943. (*c*) Lesser, A.: Ann. Surg. **121**:257, 1945.

2. (*a*) Blackwood, W.: Brit. J. Surg. **31**:329, 1944. (*b*) White, J.: New England J. Med. **228**:211, 1943. (*c*) White, J., and Warren, S.: War Med. **5**:6, 1944.

3. Davis, L.; Scarff, J.; Rogers, M., and Dickinson, M.: Surg., Gynec. & Obst. **77**:561, 1943.

4. McGovern, T.; Wright, I., and Kruger, E.: Am. Heart J. **22**:583, 1941.

5. Ducuing, J.; D'Harcourt, J.; Folch, A., and Bofil, S.: J. de chir. **55**:385, 1940.

6. Larrey, D. J.: Mémoires de chirurgie militaire et campagnes, Paris, C. J. Smith, 1812, vol. 3, p. 60.

7. (*a*) Patterson, R. H., and Anderson, F. M.: Surg., Gynec. & Obst. **80**:1, 1945. (*b*) Abramson, D.; Lerner, D.; Shumacker, H., and Hick, F.: Am. Heart J. **32**:52, 1946. (*c*) Leigh, O.: Ann. Surg. **124**:301, 1946. (*d*) Boland, F.; Claiborne, T., and Parker, F.: Surgery **17**:564, 1945. (*e*) Paddock, F.: New England J. Med. **34**:433, 1946. (*f*) Osborne, J., and Cowen, J.: Lancet **2**:204, 1945. Friedman.[1a] Lesser.[1c]

The men complained of pain on walking and of hyperhidrosis. Often a patient alleged that he had to change his socks two or three times daily because of perspiration. The pain was located primarily in the balls of the feet, and as a result the men refused to put any weight on this part of their feet and walked with a characteristic shuffle. On arrival at the general hospital, they did not cover their feet with blankets or sheets, claiming that covering caused their feet to burn. This difficulty disappeared spontaneously within a few weeks. Many of the men described increased sensitivity to marked changes in temperature as compared with what they had experienced earlier in life.

What was most surprising was the contrast between the complaints of men who had lost tissue and those who had not. The latter were much more vociferous in complaining of their symptoms, especially of the hyperhidroses and pain.[8] On the other hand, it was not at all unusual to see a man with several gangrenous toes getting about with little complaint and even playing softball.

In contrast to the subjective symptoms, objective findings were lacking in the men who did not suffer loss of tissue. The pedal hyperhidrosis was accompanied almost invariably by manual hyperhydrosis. A study, in the same hospital, of the pulses and sweat responses failed to demonstrate any clearcut differences between a large group of patients with trench foot and other hospital patients not suffering from any peripheral vascular disease.[8a] Some cyanosis and edema were apparent on standing but were never marked enough to use as a differentiating point in diagnosis. The diagnosis of trench foot at this stage was based purely on the history.[8b]

Other investigators [9] have studied the clinical aspects of the disease and have, in general, encountered the same clinical symptoms. Stein and Dry [9a] have noted that no tests indicated any organic disease but that some tests suggested the possibility of functional derangement. Abramson and associates [7b] apparently depended primarily on the patients' subjective complaints. In a follow-up of their cases, Abramson and Shumacker [9b] concluded that sympathectomy was of no demonstrable value. Boland and co-workers [7d] were unable to distinguish between patients and controls with regard to skin reactions induced by changes of

8. (*a*) Silverman, J.: Ann. Int. Med. **22**:702, 1946. (*b*) Krause, L.: Personal communication to the author.

9. (*a*) Stein, I., and Dry, T.: Mod. Conc. Cardiovasc. Dis., 1946, vol. 15, no. 9. (*b*) Abramson, D., and Shumacker, H., in Proceedings of the Eighteenth Annual Meeting of the Central Society for Clinical Research, Chicago, Nov. 2-3, 1945. (*c*) Patterson and Anderson.[7a] (*d*) Abramson and others.[7b] (*e*) Leigh.[7e] (*f*) Boland and others.[7d] (*g*) Paddock.[7e] (*h*) Osborne and Cowen.[7f]

temperature in the more chronic stages of the disease. Paddock [7e] noted certain minimal changes in temperature reactions, but these could have been due to a preexisting neurovascular imbalance which in itself would predispose toward susceptibility to trench foot.

In the men who had lost tissue, the areas immediately adjacent to the demarcation zone were fibrous, with splotchy cyanotic and erythematous areas. At amputation the surgeons often remarked about the lack of elasticity of the tissues. However, beginning about 3 to 4 cm. proximal to the demarcation zone, the tissues appeared grossly normal.

Characteristically, the symptom of pain in the balls of the feet was relieved when the men were induced to walk correctly. A typical example is case 183928. For about two years the patient had refused to place any weight on the ball of his right foot and had walked with a marked limp somewhat similar to that seen in a case of peroneal palsy. When he was reassured and began to walk correctly, a tremendous improvement was apparent in a few days. The edema of other patients who had consistently refused to get out of bed was also relieved when they were persuaded to walk.

MATERIAL AND METHODS

Of the 15 patients studied, 7 had lost tissue varying from a part of a toe to two or three phalanges of all the toes. The amputation specimens will be made the subject of another report. In the remaining 8, whose involvement was of the milder type, the gangrenous tissues had been exfoliated without any permanent loss of tissue.

All of the patients were combat soldiers. Fourteen had been exposed to cold wet weather in the winter of 1944-1945 in the European Theater of Operations. One (183928) had received his injury in the Attu campaign of 1943, and biopsy was done about two years after his evacuation.

Control biopsy specimens were obtained from volunteers, none of whom had been overseas. All had passed recent physical and laboratory examinations, preparatory to entering medical school. They were in the same age group as the patients, but there was not as great a spread between the youngest and the oldest as among the patients.

The biopsy tissues were obtained from two locations—the inner side of the foot medial to the phalangeal-metatarsal jonit and the lower one fourth of the leg medial to the tibia. Each specimen included epithelium, dermis and a small amount of subcutaneous tissue but no muscle. Each patient, as well as each control, was kept in bed for at least ten hours prior to biopsy. Each had the same preanesthetic medication (morphine and atropine) and was operated on under intravenous pentothal anesthesia by the same surgeon.

The tissues were fixed for from four to six hours in Helly's fluid, embedded in pyroxylin (nitrocellulose) and cut serially at 8 to 10 microns. All tissues were stained with hematoxylin-eosin-azure II, Mallory-azan and orcein. In addition,

many of the slides were impregnated with silver by a modified Foot technic for reticular fibers.[10] Specific nerve stains or impregnations were not done.

MICROSCOPIC DESCRIPTION

Tissues from Controls.—Because certain characteristics thought of as abnormal if encountered elsewhere in the body were found to be present in the controls, a histologic description of the tissues of the normal foot seems warranted in order to emphasize these unusual phenomena. Another important point noted was the variability of a structure from person to person and even in the same person. This included almost any of the structures—for example, the number of sweat glands or the amount of collagen in a nerve (figs. 8 and 9).

The epidermis was of the usual type, with the four classic layers fairly well marked. The size and the number of melanin granules in the basal germinative layer varied, as did also the amount of intercellular and extracellular fluid. Mitoses were seen only once or twice in slides of the eight biopsy specimens. No sign of leukocytic invasion of the epidermis was seen in any control.

Degenerated cells were extremely rare. There was some variation in the number, the size and the depth of the rete pegs from control to control and even in the same control. In none were they absolutely uniform in size, shape and frequency. The epidermis was always separated from the underlying papillary layer by a clearcut reticular fiber basement membrane.

Neither the size nor the number of sweat glands was uniform, and the amounts of secretory granules and especially the height and the staining quality of the secretory cells were subject to wide variations. Basophilic granules filled the lumen and the distal ends of some cells and were entirely lacking in others. The nuclei were of different heights and degrees of turgidity. There was usually much less variation in the duct cells and in the numbers of myoepithelial cells and vessels in the fatty connective tissue supporting these glands.

The connective tissue elements, vessels, cells and fibers, were always subject to wide variation from control to control and often in the same slide.

The number, the size and the dilatation of the papillary capillary loops were extremely variable. In some instances, papilla after papilla was filled with distended capillary loops. The ascending and descending loops were always identical morphologically, and it was impossible to determine which was the ascending and which the descending loop except by use of serial sections. The reticular fiber network of the subpapillary arteries and veins continued over on the papillary capillary loops. The elastic fibers never followed the vessels superficially to the subpapillary plexus and in the papillae had no direct relation to the capillaries of the papillae.

The arteries in the subcutaneous layer differed in size and contractility but were all qualitatively similar in that the usual layers of intima, muscularis and adventitia were quite clear. The walls of the vessels were heavy in some subjects, delicate in others. There was always a slight variation in the amount of edema and intercellular fibers between the individual smooth muscle cells of the muscularis, although there were never enough fibers in the media to make it impossible to differentiate the arteries from the veins.

The arteries of the subcutaneous plexus sent branches superficially through the reticular layer to form the subpapillary plexus. As these coursed through the

10. Buchsbaum, R., and Loosli, C.: Methods of Tissue Culture in Vitro and Outlines of Histological Methods, Chicago, University of Chicago Press, 1936.

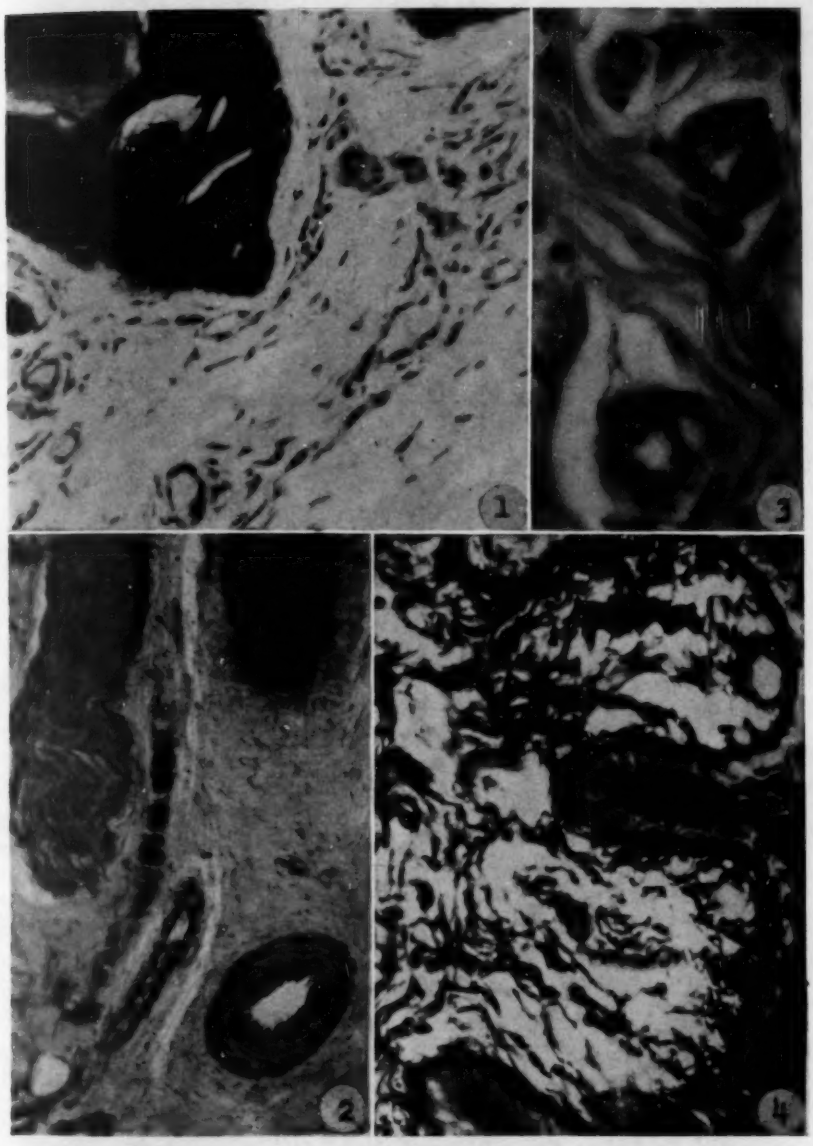
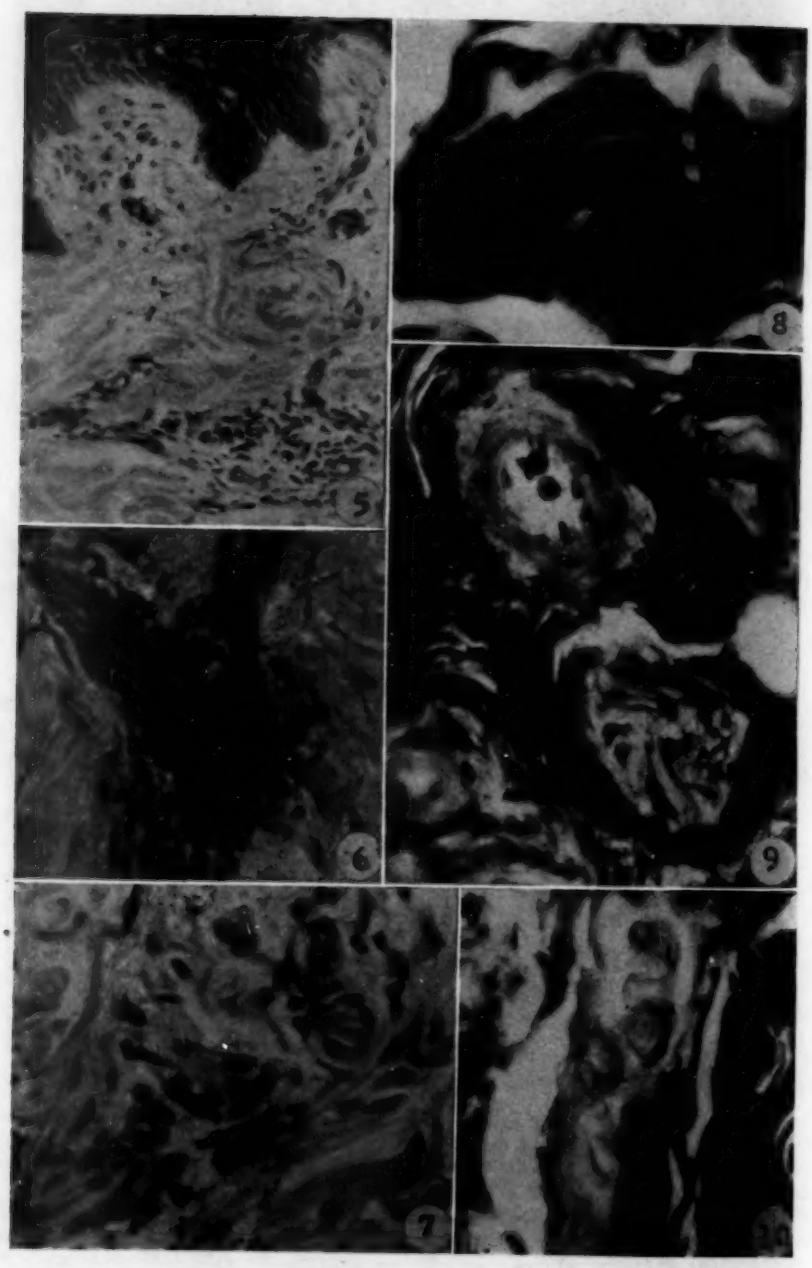


Fig. 1 (patient).—Moderate but variable amounts of epidermal intercellular edema. Marked dilatation of capillary loops in papillae and of subpapillary vessels. No inflammatory cells. Hematoxylin-eosin-azure II; × 210. Army Institute of Pathology negative 98565.

Fig. 2 (patient).—Heavy-walled subcutaneous vessels. Small amount of neural and perineural collagen. Hematoxylin-eosin-azure II; × 135. Army Institute of Pathology negative 98559.

Fig. 3 (control).—Sclerotic small artery and, below and to left, normal small artery in subpapillary plexus. Hematoxylin-eosin-azure II; × 1,000. Army Institute of Pathology negative 98569.

Fig. 4 (patient).—Normal reticular fibers in papillae with perfectly preserved basement membrane. Silver; × 500. Army Institute of Pathology negative 98615.



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reticular layer, they lost their definite adventitial coat, and the internal elastic membrane became irregular and discontinuous. In the subpapillary plexus the arteries were reduced to an endothelium and a single irregular layer of smooth muscle cells (fig. 3). Usually an internal elastic membrane was lacking, but occasionally it was still demonstrable. The reticular fiber sheath was always quite heavy, heavier than about the veins. There was a great deal of variation in the amount of sclerosis in these arteries (fig. 3).

The intima and the media of the subcutaneous veins were always mixed so that one might speak of them collectively as an intima-media. The elastic fibers were scattered through the intima-media and did not form an internal elastic membrane, but an external elastic membrane was usually quite prominent. The subcutaneous veins sent branches through the reticular layer to form several plexuses in the reticular layer and finally the subpapillary plexus. The veins of the subpapillary layer never had any smooth muscle and were recognized primarily by their reticular and elastic fibers.

The fibers forming the scaffolding of the connective tissue of the skin were heavy and numerous.

Reticular fibers were found only in certain locations. In the subcutaneous layer they formed a network about individual fat cells, sweat glands and vessels. In the reticular layer they were found only perivascularly. In the papillary layer they formed the basement membrane and capillary sheaths and were hardly to be seen at all in the rest of the papillae.

The collagenous fibers of the reticular layer were extremely heavy, dense and somewhat hyalinized (figs. 3 and 9). There were variations in staining, so that in the Mallory-azan stain a few of the fibers stained red; in most cases they consistently stained blue. There was always a contrast in the collagen of the reticular and papillary layers in that the fibers of the latter were always finer and looser and there was more interfibrillar fluid.

The elastic fibers were fairly evenly distributed in the reticular layer, but in the subcutaneous layer they were sparse. They usually adopted a corkscrew appearance in the papillary layer, between vessels and basement membrane, and also formed a network anastomosing just superficial to the basement membrane.

Numerous inflammatory cells were seen about the subpapillary plexus and about the branches of vessels coming from the subcutaneous plexus near the sub-

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Fig. 5 (control).—Perivascular inflammation. Twisting capillary loops; moderately dilated vessels. Normal collagen. × 450. Army Institute of Pathology negative 98619.

Fig. 6 (control).—Marked perivascular inflammation. Hematoxylin-eosin-azure II; × 450. Army Institute of Pathology negative 98567.

Fig. 7 (control).—Moderate perivascular inflammation. Hematoxylin-eosin-azure II; × 545. Army Institute of Pathology negative 98610.

Fig. 8 (control).—Small amount of perineural fibrosis; rather marked endoneural fibrosis. Mallory-azan. Army Institute of Pathology negative 98612.

Fig. 9 (control).—Marked perineural fibrosis. Normal small artery, although slightly dilated. From same patient as figure 8. Mallory-azan; × 545. Army Institute of Pathology negative 98613.

Fig. 10 (patient).—Minimal amount of perineural fibrosis. Average amount of collagen in endoneurium. Mallory-azan; × 660. Army Institute of Pathology negative 98611.

papillary plexus. There was a wide variation among the patients and the controls in this respect, some not having any inflammatory cells, others having so many that the areas would certainly have been called abnormal in any other circumstances (figs. 5, 6 and 7). These cells were primarily lymphocytes and monocytes, although in a few instances they were macrophages and fibroblasts. In some controls there were numerous mast cells in the same locality; in others, none at all. The granules of the mast cells were rather small and ill defined. There was no evidence of mononuclear invasion of any structure.

Another striking inconsistency was in the amount of collagenous and reticular fibers seen in the nerves (figs. 8 and 9). In one person the perineurium had so much collagen that it was almost one-third as thick as the nerve; elsewhere in the same person the perineurium was so fine as to be hardly recognizable (fig. 8). The endoneural fibers likewise varied from the finest to the coarsest. There was no correlation between the amount of fibers and any other characteristic on the slide.

There was nothing unusual in the fatty tissue of the subcutaneous layer.

Tissues from Patients.—A comparison of the biopsy specimens from patients and controls failed to reveal any significant difference between the two groups in regard to any structure examined (figs. 2, 4 and 10). However, special nerve fiber preparations were not made, and the biopsy specimens were not deep enough to include muscle. But there were pathologic features in tissues from a certain few of the patients which were not duplicated in any of the controls and which were probably related to the past history of trench foot.

In case 183435 an unusually large amount of edema was present in the papillary layer of the dermis. It is probable that the patient's vessels were unusually thick and prominent (fig. 2). There was an unusually large number of prominent vessels in the subpapillary plexus and in the connective tissue of the papillary layer of the dermis in case 183430 (fig. 1). It must be emphasized that these variations were minute, and it was only after the specimens had been compared with the controls repeatedly that the distinctions were established. The clinical courses were similar in that both patients refused to get out of bed and walk as did the other patients. The biopsy specimens were taken when both were still in bed with doughy, edematous feet, painful to palpation. Trench foot was moderate in both patients; there was no loss of tissue, and improvement was rapid after the patients began to walk.

In case 183434, in which trench foot was mild, the intima of a vein in the superficial part of the reticular layer of the dermis was greatly thickened and was quite as abnormal as any of the vessels seen in the amputated specimens. However, there was nothing unusual in any other part of this biopsy specimen.

Surprisingly enough, no pathologic changes were found in any of the specimens from the men who had lost tissue. In 1 case the second or third phalanx of each toe was amputated at the same time that the biopsy specimens were taken. The biopsy specimen taken only a few centimeters from the amputation line failed to show any pathologic features. Apparently, the marked pathologic changes occurring in the zone of regeneration of the amputated specimen did not extend proximally far enough to involve the site of removal of biopy tissues in any of the patients.

In both patients and controls occasionally there were certain morphologic features which were characteristic enough to serve as a means of identifying the source of the tissues. For example, one man had extremely heavy-walled arteries and veins. These structures were all large and gross as compared with those of any of the other men (fig. 2). Some of the men had more pigment in the stratum germinativum of the epidermis than others. There were variations in the number

and the prominence of vessels from one man to the next. In brief, no two specimens were exactly alike, although, as mentioned previously, the differences were too inconsistent to serve as a means of separating the controls from the patients.

COMMENT

In previous reports of biopsies of the chronic stages of trench foot and immersion foot, certain pathologic features have been described. Boland and associates [7d] described vascular dilatation, perivascular inflammation and increase of collagen occurring about ninety days after the onset of symptoms as compared with control tissue taken post mortem from patients with previously normal feet. White and Warren [2c] noted increased thickening of the corium, scarcity of skin appendages, hyalinization of collagen, smoothing of rete pegs, perineural fibrosis and fibrosis extending along individual muscle fibers and groups of fibers. Paddock [7e] noted minor swellings of axons and minimal neural fibrosis. Ungley and Blackwood [11] described changes in the larger nerves and vessels. However, these investigators [12] did not have the opportunity to obtain control biopsy specimens from the same location in healthy living normal persons and so were not able to take into account the specialized structure of tissues of the feet. Unfortunately, none of my biopsy specimens was taken deep enough to contain muscle, and so no opportunity was available to study the deeper structures.

Blackwood and Russell,[13] after observing experimental lesions of rats simulating trench foot, emphasized the deep neural and muscular changes and failed to find anything significant in the chronic stages as far as the blood vessels were concerned. Denny-Brown and co-workers [14] stated that the neural lesions produced experimentally under conditions similar to those causing trench foot tend to undergo complete repair within ninety days.

In regard to pernio, McGovern, Wright and Kruger,[4] from their observations of biopsy material, described changes in the vessels and connective tissues which were similar to, if not identical with, those seen in the amputated specimens from patients with trench foot. However, the appearance of the tissue in their figure 16 seems within normal range.

The minimal, perhaps debatable, pathologic changes encountered in specimens from 2 patients with a mild form of trench foot should not be

11. Ungley, C. C., and Blackwood, W.: Lancet **2**:447, 1942.

12. White and Warren.[2c] Boland and others.[7d] Paddock.[7e] Ungley and Blackwood.[11]

13. Blackwood, W., and Russell, H.: Edinburgh M. J. **50**:385, 1943; **52**:160, 1945.

14. Denny-Brown, D.; Adams, R. D.; Brenner, C., and Doherty, M.: J. Neuropath. & Exper. Neurol. **4**:305, 1945.

interpreted in the same light as the changes in tissues from the other patients. By refusing to walk, these 2 patients retarded the normal healing processes; thus the same stage in repair had not been attained as in the tissues of the men who were already actively ambulant at the time of biopsy.

One objection may be raised to the method by which the biopsy tissues were taken. Since both patients and controls were kept in bed for the ten hours prior to operation, any latent deficiency of the functioning of the neurovascular system would have been masked in the same way as resting in bed will prevent distention of varices in varicose veins of the lower limb. Perhaps if the controls and the patients had been made to walk up to the moment the biopsy specimens were taken, the tissues might have been more edematous and the vessels more dilated. But the strain occasioned by walking would hardly have been expected to change the basement membrane or the nerves.

The interpretation of the functional state of the patients in terms of the biopsy results was extremely difficult. Certainly, neither the biopsies nor the objective clinical findings revealed anything at all which could explain the clinical symptoms. The speed with which these symptoms were alleviated indicated that little of pathologic significance would be found after the men had been ambulant for some time. However, it is realized that absence of microscopic lesions does not exclude the possibility of functional derangement. Furthermore, the excision of specimens was not deep enough to exclude deep nerve and muscle disease, although the symptoms and the clinical course were such as not to point to changes of muscle or deep tissues.

The individual differences found from patient to patient and even in the controls were interesting. It seemed as though no two skins were absolutely identical. In the same way the individual reactions to cold wet weather were variable. In only a small percentage of any unit did trench foot develop. Some men were obviously more susceptible than others to the environmental changes.[7a, f] In a report of carefully controlled animal experiments, Böttcher[15] has referred to the marked individual variation in susceptibility of rats to cold. Since there were morphologic variations in the controls, it seems quite possible that there may also be physiologic variations in the neurovascular system, and perhaps both of these will serve to indicate in the future who will and who will not be an early candidate for trench foot.[7f]

SUMMARY

Biopsies were performed on 15 men who had absolutely clearcut histories of uncomplicated trench foot. Of these 15 men, 7 had lost some

15. Böttcher, H.: Virchows Arch. f. path. Anat. **312**:464, 1944.

tissue. The biopsy specimens from these patients as a group failed to reveal any pathologic changes when compared with those from 8 controls of the same age group.

Certain morphologic features—mild hyalinization of nerves, perineural fibrosis, perivascular inflammation and hyalinization of collagen—which would ordinarily be considered abnormal were found in both patients and controls.

Many of the individual subjects exhibited slight variations from the average which indicated the possibility that variation may occur in physiology and consequently in susceptibility to environmental changes.

In treatment the best results were obtained by inducing the patients to walk as early as possible.

PATHOLOGIC SIGNIFICANCE OF THE DUCTUS ARTERIOSUS

Its Relation to the Process of Arteriosclerosis

LESTER S. BLUMENTHAL, M.D.*
ROCHESTER, MINN.

A SURVEY of mortality rates of recent years [1] reveals that diseases of the heart and the blood vessels now head the list of causes of death. The primary unsolved problem in pathology continues to be that of arteriosclerosis.[2] This condition serves as a basis for most of the clinical diseases of the heart and the blood vessels. With this in mind, I have studied a small aspect of the gross problem.

One of the vascular structures of the body where a phenomenon resembling arteriosclerosis of later life develops early is the fetal ductus arteriosus, closure and subsequent obliteration of which occur soon after birth. Robertson [3] expressed the opinion that the closure of the ductus arteriosus is a form of arteriosclerosis and that in the processes of its closure some processes that resemble arteriosclerosis of later life might be observed. Winternitz and his associates [4] studied the "adaptive" changes that occur normally in three groups of blood vessels. These changes are the obliteration of the fetal vessels at birth, the changes in the uterine artery during and after pregnancy, and the obliteration of large pulmonary vessels that traverse tuberculous cavities. He demonstrated increased vascularity of the walls of the vessels in these "adaptive" changes, together with many other changes, including hemorrhage and calcification, which he found in arteriosclerotic vessels. He thought this demonstration increased the significance of the vascularity of arteriosclerotic sites and emphasized anew the validity of the idea that pathologic changes have their less violent counterparts in normal physiologic processes.

The implied relationship between the closure of the ductus arteriosus and the process of arteriosclerosis led to the present investigation, to

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* Fellow in Medicine, Mayo Foundation.

1. Twenty-Five Years of Health Progress, New York, Metropolitan Life Insurance Company, 1937.

2. Arteriosclerosis, editorial, J. A. M. A. **128**:517, 1945.

3. Robertson, H. E.: Personal communication to the author.

4. Winternitz, M. C.; Thomas, R. M., and LeCompte, P. M.: The Biology of Arteriosclerosis, Springfield, Ill., Charles C Thomas, Publisher, 1938.

see whether there is a parallel between arteriosclerosis and the closure of this vessel and to determine whether any further information can be obtained about the etiologic nature of arteriosclerosis.

THE DUCTUS ARTERIOSUS

The ductus arteriosus has received attention recently because heroic surgical procedures have been devised to correct persistent patency of the vessel.[5] The literature contains numerous excellent reports dealing with the manner of the normal closure at birth. These include embryologic,[6] anatomic,[7] histologic[8] and physiologic studies.[9] Most of the investigations were undertaken to substantiate one or another theory of the closure of the ductus; however, none attempted to correlate this process with the arteriosclerosis of later life.

5. Gross, R. E.: J. A. M. A. **115**:1257, 1940. Gross, R. E., and Hubbard, J. P.: ibid. **112**:729, 1939. Harrington, S. W.: Proc. Staff Meet., Mayo Clin. **18**:217, 1943. Keys, A., and Shapiro, M. J.: Am. Heart J. **25**:158, 1943. Touroff, A. S. W.: ibid. **25**:187, 1943.

6. (*a*) Arey, L. B.: The Vascular System, in Developmental Anatomy: A Text-Book and Laboratory Manual of Embryology, ed. 3, Philadelphia, W. B. Saunders Company, 1934, chap. 11, pp. 280-331. (*b*) Congdon, E. D.: Contrib. Embryol. **14**:47, 1922. (*c*) Gray, H.: Anatomy of the Human Body, ed. 23, edited by W. H. Lewis, Philadelphia, Lea & Febiger, 1936. (*d*) Heuser, C. H.: Contrib. Embryol. **15**:121, 1923.

7. (*a*) Cunningham, D. J.: Text-Book of Anatomy, ed. 8, New York, Oxford University Press, 1943. (*b*) Eaton, A. W.: Unpublished data. (*c*) Gräper, L.: Ztschr. f. Anat. u. Entwcklngsgesch. **61**:312, 1921. (*d*) Jager, B. V., and Wollenman, O. J.: Am. J. Path. **18**:595, 1942. (*e*) Morris, H.: Morris' Human Anatomy: A Complete Systematic Treatise, ed. 10, edited by J. P. Schaeffer, Philadelphia, The Blakiston Company, 1942. (*f*) Noback, G. J., and Rehman, I.: Anat. Rec. **81**:505, 1941. (*g*) Roeder, H.: Arch. f. Kinderh. **33**:147, 1902. (*h*) Strassmann, P.: Arch. f. Gynäk. **45**:393, 1894. (*i*) Gray.[6e]

8. (*a*) Barnard, W. G.: St. Thomas's Hosp. Rep. **4**:72, 1939. (*b*) Boyd, J. D.: J. Anat. **75**:457, 1941. (*c*) Costa, A.: Cuore e circolaz. **14**:546, 1930. (*d*) da Cunha, M. L.: Fac de med. de São Paulo **2**:245, 1927. (*e*) Kennedy, J. A., and Clark, S. L.: Anat. Rec. **79**:349, 1941. (*f*) Mĕlka, J.: Anat. Anz. **61**:348, 1926. (*g*) Swensson, A.: Ztschr. f. mikr. anat. Forsch. **46**:275, 1939. (*h*) Takino, M., and Watanabe, S.: Arch. f. Kreislaufforsch. **2**:18, 1937. (*i*) von Hayek, H.: Ztschr. f. Anat. u. Entwcklngsgesch. **105**:15, 1935. (*j*) Footnotes 7 *b*, *c* and *d*.

9. (*a*) Barclay, A. E.; Barcroft, J.; Barron, D. H., and Franklin, K. J.: Brit. J. Radiol. **11**:570, 1938; (*b*) **12**:505,1939. (*c*) Bettinger, H.: Centralbl. f. allg. Path. u. path. Anat. **54**:289, 1932. (*d*) Kennedy, J. A.: Am. J. M. Sc. **204**:570, 1942. (*e*) Kennedy, J. A., and Clark, S. L.: Am. J. Physiol. **136**:140, 1942. (*f*) Kowalski, W.: Virchows Arch. f. path. Anat. **233**:191, 1921. (*g*) Linzemeier, G.: Ztschr. f. Geburtsh. u. Gynäk. **76**:217, 1915. (*h*) Rauchfuss, C.: Virchows Arch. f. path. Anat. **17**:376, 1859. (*i*) Rehman, I.: Abstracted, Anat. Rec. (suppl., no. 2) **76**:47, 1940. (*j*) Schanz, F.: Arch. f. d. ges. Physiol. **44**:239, 1889. (*k*) Wells, H. G.: Am. J. M. Sc. **136**:381, 1908. (*l*) Footnote 7 *b*, *c* and *d*. (*m*) Footnote 8 *a*, *e*, *f*, *g* and *i*.

Kennedy and Clark [9e] presented a detailed description of the vasa vasorum of the ductus arteriosus and found these to be present in the outer portion of the media at birth. That portion of the wall of the ductus that lacked these vessels was the same portion in which the most marked degenerative changes occurred. They therefore postulated that after physiologic closure of the lumen of the ductus the central part is no longer able to obtain a supply of blood and that, because of this, degeneration occurs.

MATERIAL AND METHODS

The material of the present study was obtained from a series of 175 postmortem specimens. The specimens were obtained from fetuses and from persons of various ages. The oldest was a man aged 96 years. The ductus arteriosus or the ligamentum arteriosum arteriae pulmonalis was removed intact, fixed in solution of formaldehyde U. S. P., sectioned, and stained with hematoxylin and eosin. Many sections were also stained with the Van Gieson or with the elastin H stains to demonstrate more clearly the elastic, muscular and connective tissues.

RESULTS

The only practical way to present the findings is to describe specimens representative of each age group. The specimens representing fetuses in the earlier stages of development had the basic structure found in those representing fetuses at full term. There was little change in this until after birth. From birth to the first few weeks of life the most extreme alterations were observed. By that time degeneration had begun. The only change that occurred later was an increase of the degeneration of the tissues, but even with definite degeneration the basic structure of the ductus could be distinguished with ease.

Fetuses.—The youngest fetus was just past the fifth month. The cross section of the ductus arteriosus showed a layer of normal endothelium with a single-layered internal elastic lamina below. Immediately adjacent to this there was wide-meshed areolar-like tissue. This was occupied in certain locations by wandering cells and by dark-staining small spindle-shaped cells lying in a direction perpendicular to the internal elastic layer. Deeper in this areolar-like tissue the smooth muscle cells of the media at first appeared separated by tissue spaces and tiny fibrils, but as the adventitia was approached, their arrangement became more compact and assumed a definitely circular aspect. The outer layers of the media blended imperceptibly with the adventitia, without an intervening external elastic lamina. The media was strikingly free from the thick bands of elastic tissue that are seen in the pulmonary artery and the aorta.

In some specimens there were elevated plaques or mounds such as are seen grossly when the intima of a patent ductus arteriosus is examined at birth. These mounds are often made up of cells which closely

resemble the smooth muscle cells of the media, and there seems to be a "streaming in" of these cells from the media to the mounds. Where the lumen was reduced in diameter, the mounds were higher and more compact. The media also was more compact, and the tissue spaces between the muscle cells were much narrower. This probably was evidence of active muscle contraction.

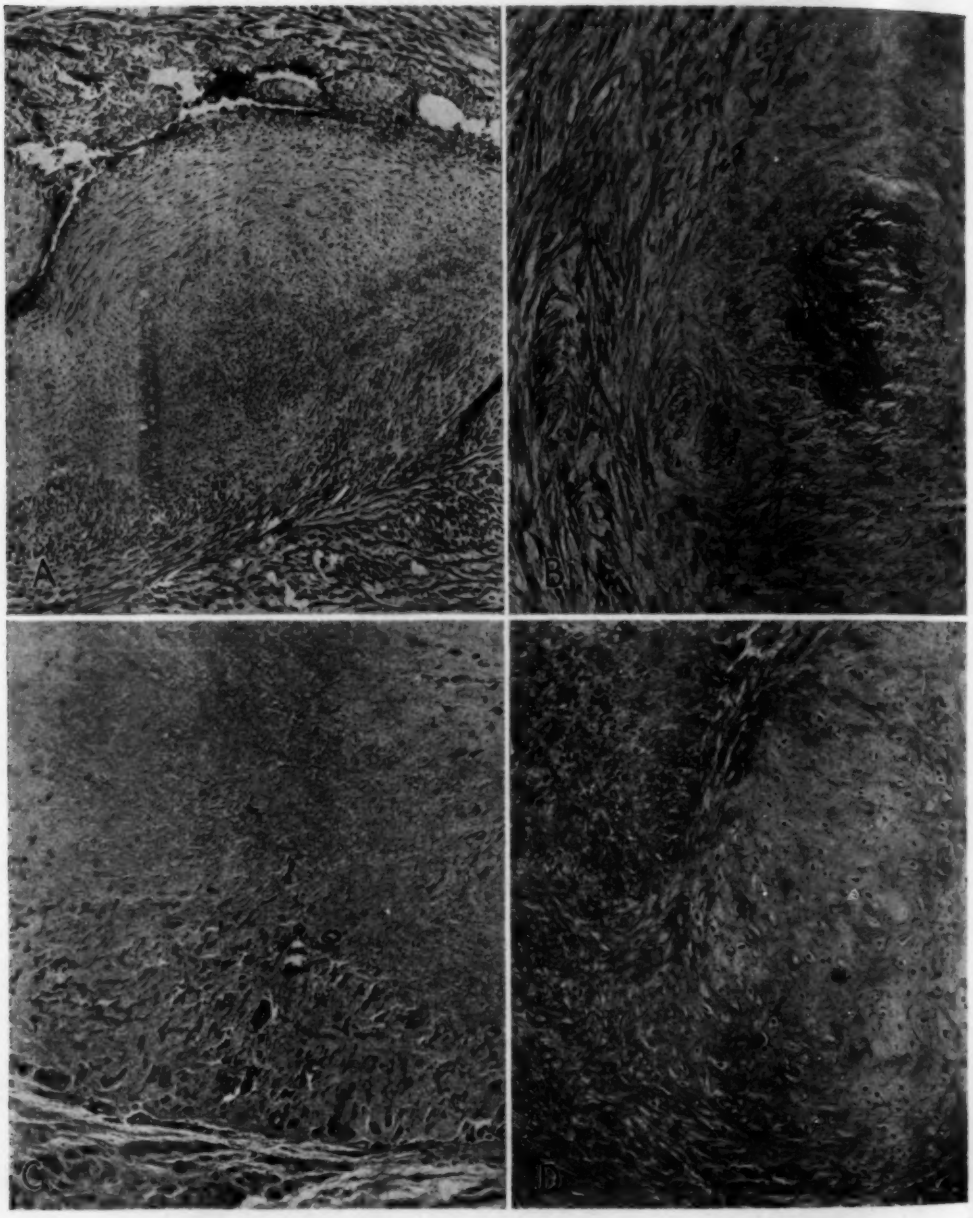
Infants from Birth to Twelve Days of Age.—The wall of the duct was the same in this group as in the premature group. The mounds were more numerous and larger. The internal elastic layer was split and reduplicated into two or three thin strands. The media was composed of thicker bundles of circular and oblique muscle cells, with wide tissue spaces between these bundles. There was no evidence of degeneration in any specimen. The tissue of the mounds and the smooth muscle of the media stained yellow with the Van Gieson stain and contrasted markedly with the red-staining fibrous connective tissue of the adventitia.

Infants from Twelve Days to Two Months of Age.—This group, which included specimens from 8 infants, was the most interesting to observe. A gradual progression of degenerative changes took place in the ductus arteriosus, beginning on the twelfth day. On the twelfth day the lumen of the ductus was contracted to a narrow slit. There was a ghostlike washed-out appearance, as if infarction had occurred in the intima and the inner part of the media.

The specimen from the infant 16 days old (*A* in figure) showed the tissue to be compressed, with almost complete obliteration of the tissue spaces of the mounds. There were a decrease in the number of deep-staining nuclei, an increase in the amount of elastic fibrils and marked ischemia of the entire mound. When Van Gieson stain was applied, the tissue took on a pink or a red tint, which is evidence of fibrosis. There was no actual necrosis or deposition of calcium.

The next step in the degenerative process was seen in the specimen (*B* in figure) from an infant 25 days old. Generalized washed-out appearance and lack of nuclei were observed in the cells of the intima and the inner part of the media. The muscle cells were paler, and much fibrous connective tissue was interspersed among them. One area showed early necrosis of the intima.

As the age increased, the area of ischemia increased, and more fibrous connective tissue replaced normal muscle tissue. This process of degeneration gave the impression that as the ductus contracts the imbibition of blood and plasma is decreased with resultant ischemia of the tissues. If the degeneration progresses slowly, the specialized smooth muscle is gradually replaced by fibrous connective tissue. It may be that this ischemia acts as a stimulus for the elastic tissue to disrupt or split up rather than to degenerate, probably because elastic tissue is a



Cross sections of ligamentum arteriosum stained with hematoxylin and eosin: *A*, from an infant 16 days of age (× 65); *B*, from an infant 25 days of age (× 150); *C*, from a woman 22 years of age (× 110); *D*, from a man 56 years of age (× 80).

less specialized tissue than muscle tissue and therefore needs less oxygen to exist. If the ischemia is excessive, there may be actual necrosis of tissue instead of replacement by tissue of a lower order.

Infants from Two Months to One Year of Age.—The degenerative changes continued to increase. Most specimens contained areas of necrosis and calcium deposits. The tissue adjacent to the slitlike remnants of the lumen appeared to be new and alive, and many red blood cells in good state of preservation remained in the lumen. Small capillaries were often seen adjacent to the lumen. These may represent efforts to recanalize the tissue. The outer layers of muscle retained their normal appearance, but isolated nuclei and an occasional clump or bundle of muscle cells were all that remained of the inner layers.

After the Age of One Year.—Van Gieson stain showed a gradual increase of the intensity of the fibrosis until the color of the new tissue almost matched the deep red–staining fibers of the adventitia. The only significant change was the decrease in smooth muscle at the outer part of the media. This gradually became reduced to single strands in a circular manner and definite clusters of cells in certain areas. After many sections presenting the same cluster-like arrangement of these muscle cells were observed it became evident that there was a definite reason for this arrangement. It was found that these clusters were adjacent to small blood vessels and that the clusters were usually absent in areas devoid of capillaries. *C* in the figure demonstrates this arrangement well. These findings give support to the idea that the process of fibrous replacement of the ductus arteriosus is of an ischemic or anoxemic nature. The internal elastic lamina remained intact and prominent even in the older specimens. Elastic fibrils gradually increased in number. Before the tenth year the fibrils invaded the intima, but later they also extended throughout the media.

Another interesting finding in several specimens from older persons was formation of cartilage. *D* in the figure shows this cartilage well. The specimen was obtained from a man aged 56 years. The oldest persons from whom specimens were obtained were 90 and 96 years of age. Extreme degeneration and much deposition of calcium were observed in these two specimens, but under low magnification the basic structure was plainly evident.

COMMENT

The primary purpose of this study was to try to add to the understanding of the genesis of arteriosclerosis.

The closure and subsequent obliteration of the ductus arteriosus is a phenomenon occurring naturally in most newborn mammals. This process is a form of arteriosclerosis that occurs with a physiologic purpose. It was therefore believed that a study of the histologic changes

involved in this metamorphosis might disclose certain processes parallel to those that occur in arteriosclerosis of later life.

Most of the authors who have studied arteriosclerosis from the standpoints of pathology and etiology carefully agree in their descriptions of the histologic changes involved but do not agree in their conceptions of the etiologic factors. They are unwilling to concede that arteriosclerosis is an unavoidable accompaniment of the aging process, for to do so would be fatalistic and would make the ultimate prognosis hopeless. Most authors agree that processes which accompany the wear and tear of life play a large part. Others believe that infections and their sequelae produce the underlying change. Still others stress blood lipids as the primary causative agent.

Hueper [10] presented an excellent review of the entire subject and proposed that anoxemia is a basic means through which any or all of the various agents may work to produce arteriosclerosis. According to this concept, localized low blood pressure leads to stagnation of the blood flow and thus to anoxemia, whereas elevation of the blood pressure is accompanied by vasoconstriction which reduces the blood flow to the vasa vasorum. He did not discuss the closure of the ductus arteriosus, but it has been shown in the present study that such a mechanism could also be active here. Kennedy and Clark [9e] touched on this concept after studying the vasa vasorum of the walls of the ligamentum arteriosum.

New surgical technics for the repair of persistent patency of the ductus arteriosus have stimulated much experimental investigation of the ductus. This has added new meaning to the older histologic descriptions of the prenatal and the postnatal state of the ductus. As a result of recent physiologic and roentgenographic studies, the closure of the ductus at birth has taken on a dynamic character, for now the vessels can be seen to contract and reexpand in response to various stimuli. This proof of actual muscular contraction allows observers to divide the closure of the vessel into two separate parts. The first is the active contraction of the muscle of the media at birth. The second is the maintenance of this state of contraction after birth when a definite sequence of histologic changes occurs. The changes probably begin soon after birth. Once they have occurred, the lumen will remain contracted even if the muscle of the media has a tendency to relax.

Histologic examination of the ductus arteriosus before birth reveals that it is a true muscular organ. The intimal mounds are probably composed of smooth muscle of a modified type. Examination of the vessel after birth shows that active muscle contraction has occurred.

Twelve days after birth an interesting series of degenerative changes occurs. It is instructive to follow this metamorphosis from the actively

10. Hueper, W. C.: Arch. Path. **38**:162, 245 and 350, 1944.

functioning ductus to the obliterated degenerated ligamentum arteriosum. It seems as if actual infarction has occurred in the intima and the media. It may be that the fetal ductus receives its blood supply from its lumen and its vasa vasorum and that after active muscle contraction of the wall of the ductus has been maintained for an appreciable time, this blood flow decreases to such an extent that actual anoxemia occurs. Thereafter, smooth muscle is gradually replaced by fibrous connective tissue and elastic tissue. Another finding that points to anoxemia is the necrosis and calcification occurring in certain specimens, beginning with the specimen from an infant aged 25 days. Most likely, the usual sequence is a gradual replacement of the muscle tissue by tissue of a lower order, but if more severe anoxemia occurs necrosis results.

These findings seem to lend support to Hueper's contention that anoxemia is the fundamental factor in the genesis of arteriosclerosis. The theories of wear and tear, senescence, toxicity, infection or hyperlipemia as causative agents in arteriosclerosis are considered only briefly in this report.

SUMMARY AND CONCLUSIONS

The ductus arteriosus is a true muscular organ which on proper stimulation is capable of contracting strongly enough to obliterate its lumen. The stimulation is apparently the increase of the oxygen saturation of the arterial blood brought about by the onset of respiration in the newborn infant.[9d] After the contraction has been maintained for several days, actual histologic changes take place. Muscle tissue is replaced by tissue of a lower order of specialization. In some cases, the replacement is slow, and no necrosis occurs; however, in others rapid degeneration with necrosis and calcification takes place. The cause of this tissue replacement and degeneration appears to be relative anoxemia of the wall of the ductus brought about by contraction of the muscle. This contraction narrows the lumen, compresses the vasa vasorum and thus decreases the blood supply of the vessel wall.

These tissue changes occurring in the ductus arteriosus as the result of anoxemia seem to be similar to those observed by Hueper in arteriosclerosis. The work recorded in this paper, therefore, leads to the opinion that anoxemia plays a large part in arteriosclerotic processes and may possibly be the fundamental mechanism through which the various causal agents bring about arteriosclerotic changes.

Case Reports

PRIMARY MESOTHELIOMA OF THE PERICARDIUM

WILLIAM J. REALS, M.D., B. CARL RUSSUM, M.D.
AND
EDMUND M. WALSH, M.D.
OMAHA

THE RARITY of primary neoplasms of the heart and of its coverings, as well as the confusing clinical aspects of a patient with a tumor diagnosed as primary mesothelioma of the pericardium, led us to review the literature and to present a detailed clinical and postmortem report of a single case.

In the literature the terminology of this type of tumor is confused. Most European authors have referred to tumors of this type as "celothelioma," while Ewing [1] classified them as endothelioma, Yater [2] as endothelial carcinoma, and Geschickter [3] as mesothelioma. Klemperer and Rabin,[4] in reviewing tumors of mesodermal origin, stated that Krumbein (1924) found 30 different names for such tumors in medical literature.

The splanchnocele of the embryo gives rise to the serous cavities (peritoneal, pleural and pericardial) which are lined by persisting celomic epithelium, the mesothelium. In derivatives of the splanchnocele, celomic epithelium tends to persist, and the underlying mesoderm tends to form vascular connective tissue. Tumors arising from the pleura, the peritoneum and the pericardium, to which the name "mesothelioma" is given, show similar tendencies, with epithelial and fibrous constituents. In this paper, therefore, such tumors will be called mesothelioma.

Only 22 cases of mesothelioma of the pericardium have been recorded in medical literature. McDonald,[5] in presenting a case, credits Marchiafava (1875-1876) with recognizing and reporting the first example and cites cases of Dietrich, Okasaki, Ceelen, Sebastianoff and Winogradoff and Ervingham. Geschickter [3] reported 1 case and cited 11 collected by Bodon [6] (Chajutin, Redtenbacher, Godel, Schoppler, Loos, Hill, Tobiesen, Williams, Lazarus, Drysdale and Kaak). Single

From the Departments of Pathology and Medicine, Creighton University School of Medicine, and Creighton Memorial St. Joseph Hospital.

1. Ewing, J.: Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders Company, 1940.

2. Yater, W. M.: Arch. Int. Med. **48**:627, 1931.

3. Geschickter, C. F.: Am. J. Cancer **26**:378, 1936.

4. Klemperer, P., and Rabin, C. B.: Arch. Path. **11**:386, 1931.

5. McDonald, S.: J. Path. & Bact. **43**:137, 1936.

6. Bodon, G.: Frankfurt. Ztschr. f. Path. **40**:431, 1930.

cases were added by Dick,[7] who noted a case presented by ten Seldam,[8] and by Hines and Nolan.[9]

Microscopically, mesothelioma appears neither frankly endothelial nor frankly epithelial. It is cellular, with only a fine reticular supporting stroma, and the cells are arranged in bundles with a slight tendency toward whorl formation. Individual cells are oval to polyhedral, with large nuclei and prominent nucleoli, and mitoses are numerous.

In Dick's[7] case there was an irregular alveolar arrangement with tumor cells lining acinous spaces, but other authors (McDonald[6] and Ewing[1]) have not mentioned this distribution, nor was it seen in our case. Vascularity, however, may be a prominent microscopic feature.

REPORT OF A CASE

A. V., a 58 year old white farmer, was admitted, May 27, 1946, to Creighton Memorial St. Joseph's Hospital on the service of one of us (E. M. W.), complaining that he had suffered from severe cough, fulness of the chest and pain of the chest on exertion for six weeks. The illness seemed to follow a "heavy chest cold" with severe, poorly localized pain in the chest. On several occasions during the two weeks before admission he had coughed up small amounts of bright red blood. A physician in his home community had made a diagnosis of pneumonia and had treated him with intramuscular injections of penicillin, considerably relieving both the pain and the cough. Dyspnea and hemoptyses had, however, become worse, and the patient was therefore hospitalized for observation.

The general systemic history revealed only that the appetite had been poor since the illness began but that there had been no loss of weight. The past history was noncontributory.

The patient was well nourished and well developed. There was no cyanosis, although forced breathing was marked. The pupils reacted normally. The throat was injected, but there were no enlarged glands or other masses in the neck. There was fulness to percussion over the lower lobe of the right lung, with decreased vocal fremitus and breath sounds. A few scattered moist rales were heard posteriorly over the lower lobe of the left lung. The cardiac apex was located at the fifth interspace in the midclavicular line; the apical and radial heart rates were 86. No murmurs or irregularities were noted. The liver, the spleen and the kidneys were not enlarged, and there were no palpable masses in the abdomen. All reflexes were within normal limits.

On admission the urine was normal; the hemoglobin, 52 per cent; the red blood cell count, 2,620,000; the white blood cell count, 11,800; the differential count, monocytes 1 per cent, lymphocytes 19 per cent and neutrophils 80 per cent. Sputum showed no acid-fast organisms.

Roentgenograms of the chest suggested that there was thickening of the pleura in the right upper lung field with pleural effusion in the right lower lung field. After withdrawal of 500 cc. of thoracic fluid two days after admission, an area of increased density in the upper lobe of the right lung suggestive of atelectasis could be seen on additional films; a similar area was noted in the right cardiophrenic angle. The right side of the diaphragm was elevated (fig. 1). The thoracic

7. Dick, J. C.: J. Path. & Bact. **47**:43, 1938.
8. ten Seldam, R. E. J.: Geneesk, tijdschr. v. Nederl-Indië **76**:2703, 1936.
9. Hines, R. E., and Nolan, D. E.: M. Bull. Vet. Admin. **10**:82, 1942.

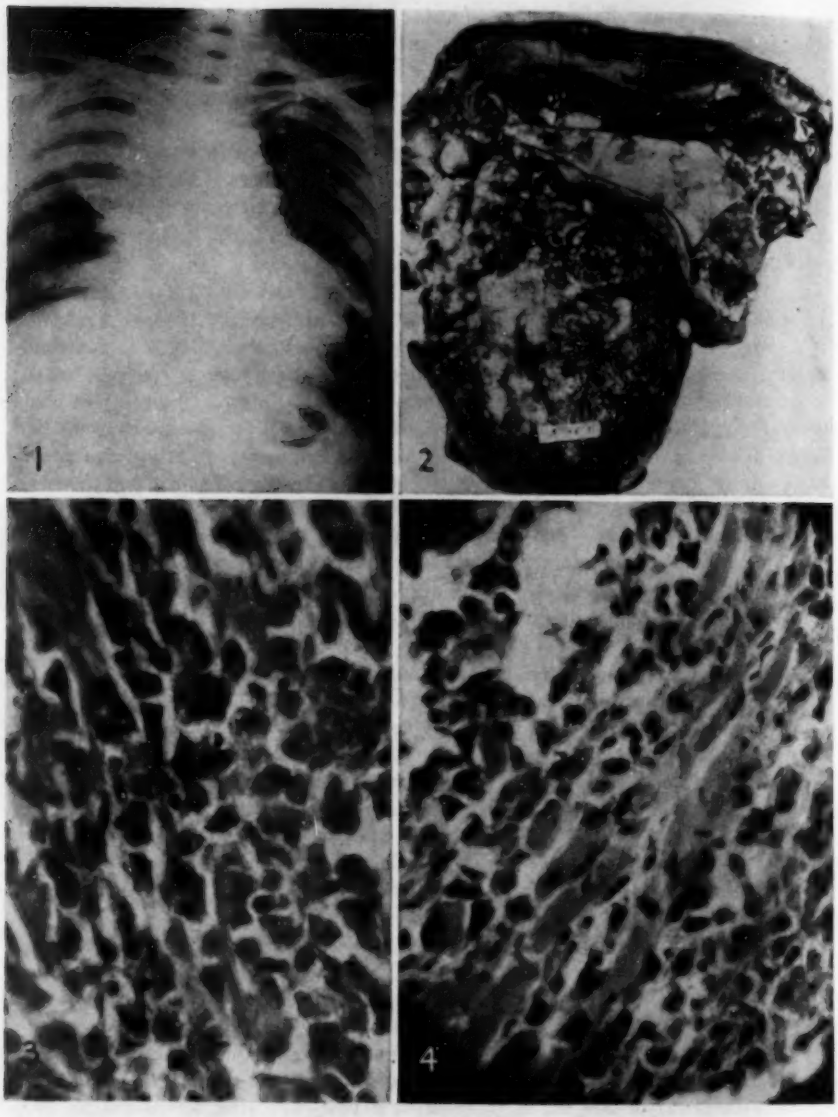


Fig. 1.—Roentgenogram of the chest. Note increased density in the right upper lung field and the right cardiophrenic angle.

Fig. 2.—Heart with the pericardium reflected upward, to show the extensive nodular involvement of the visceral and parietal pericardium.

Fig. 3.—Photomicrograph (high power) showing the pleomorphic cellular nature of the tumor of the pericardium.

Fig. 4.—Photomicrograph (high power) of the tumor's infiltration of the myocardium of the right ventricle.

fluid was dark amber in color and clear, and no growth occurred in culture mediums inoculated with it.

A provisional diagnosis of postpneumonic pleural effusion was made.

Cough was relieved with codeine. A transfusion of 500 cc. of whole blood was given. On the third day after admission the patient became suddenly dyspneic and died in twenty minutes.

Autopsy.—The principal findings were in the thorax and the abdomen. The right pleural space contained 1,500 cc. of dark amber, clear fluid; the left contained 150 cc. The pericardial sac was tense, distended, enlarged, dark blue and contained 1,000 cc. of bloody fluid. The surface of the left ventricle and the entire conus of the heart were studded with hard white tumor masses up to 3 cm. in diameter. Similar masses were attached to the internal surface of the parietal pericardium and to the pericardium over the great vessels (fig. 2). The heart weighed 450 Gm. The right ventricular surface was almost completely covered by coalescent tumor masses which invaded the heart muscle for 0.5 cm. The valve measurements were: mitral 90 mm., tricuspid 105 mm., aortic 75 mm. and pulmonary 78 mm. The right ventricular wall was 5 mm. thick and the left 15. The endocardium was unchanged. Atherosclerotic plaques were present in the root of the aorta. The coronary vessels were patent and not involved by the tumor.

The upper lobe of the right lung was partially collapsed, rubbery and dark red. The pleura over a part of the mediastinal surface of this lobe, 6 cm. in diameter, was studded with small white nodules similar to those in the pericardium. Extending from the pericardial cavity along the surface of the pulmonary artery were many small nodules 1 to 3 cm. in diameter. These masses pierced the pericardial sac and formed a large mass at the hilus, shutting off aeration of the upper lobe of the right lung and superficially infiltrating the adjacent lung tissue. The bronchi were carefully dissected, but no tumor could be found within the bronchi or the lung, although the upper right bronchus was markedly compressed. The upper lobe of the right lung was atelectatic. The remainder of the lung was not remarkable.

In the left lung there were many scattered nodules up to 2 cm. in diameter, but no areas of collapse were present. The left pleura was unchanged. The lungs together weighed 1,400 Gm. The tracheobronchial nodes were markedly enlarged; those in the right hilus contained tumor tissue as described.

The abdominal viscera were unchanged with the exception of the kidneys, which were studded throughout both cortices and medullas with small white firm tumors similar to those on the pericardium and elsewhere. The testicles, the prostate and the thyroid and adrenal glands were carefully examined for a possible primary tumor, but none was found.

Permission to examine the cranium was denied by the relatives.

The anatomic diagnosis was: primary mesothelioma of the pericardium extending locally to the myocardium, the hilus of the right lung and the pleural surface of the upper lobe of the right lung; atelectasis of the upper lobe of the right lung secondary to bronchial compression exerted by a hilar tumor mass; metastatic tumor of the left lung and both kidneys; hemopericardium; bilateral hydrothorax; generalized arteriosclerosis, grade I.

Microscopic studies were made on sections stained with hematoxylin-eosin, by Foot's method for reticulum, by the Azan method for connective tissue, and with Masson's trichrome and orcein-hematoxylin stains. Sections of the tumor and myocardium of the right ventricle revealed round to irregular tumor cells infiltrating the heart muscle, with destruction of the muscle fibers. The tumor was

highly cellular, and there was pronounced pleomorphism. The cells grew in cords with a slight suggestion of whorl formation, but no definite alveolar arrangement was seen (figs. 3 and 4). Many small blood vessels were present about the growing margin of the tumor. The cell nuclei were large with prominent nucleoli. The cytoplasm was acidophilic and finely granular. Mitoses were numerous. In sections through the hilar nodes there were areas of similar tumor cells infiltrating and destroying the lymphatic structure. Sections through tumor tissue of the pleura and underlying lung revealed masses of similar irregular tumor cells infiltrating the pleura and invading and compressing the pulmonary structure.

The supporting stroma consisted of a rather fine argentophil intercellular network. The nearby myocardium was infiltrated by polymorphonuclear leukocytes and occasional mononuclear wandering cells.

COMMENT

The diagnosis of pericardial mesothelioma must rest to a great extent on a process of elimination, in the absence of more reliable criteria. Robertson [10] cited a case in which the diagnosis was made, but subsequently the tumor was proved to be secondary to a small bronchogenic carcinoma. In our case we found no evidence that the pericardial tumor was metastatic. In sections of the bronchi taken in the area compressed by the tumor extension there was only slight atrophy of the mucosa. Cell arrangement and staining characteristics preclude the diagnosis of sarcoma of the pericardium.

McDonald [5] expressed a doubt that these tumors metastasize by the blood stream but acknowledged that lymphatic and direct extension are common. In our case, however, all forms of spread were encountered, as there were metastases in the left lung and the kidneys, direct invasion of the right hilar area and lung, and lymphatic spread to the right pleura.

SUMMARY

Mesothelioma of the pericardium is a tumor of great rarity. One case is added to the 22 recorded in the literature.

The diagnosis of mesothelioma can be made only after exclusion of all other possible primary foci.

10. Robertson, H. E.: J. Cancer Research **8**:317, 1924.

ADENOACANTHOMA OF THE ESOPHAGUS

A Report of One Case with Consideration of the Tumor's Resemblance to So-Called Salivary Gland Tumor

ELSIE McPEAK, M.D.
AND
WALTER L. ARONS, M.D.
BOSTON

CARCINOMA of the esophagus is usually epidermoid in type and in our experience this type shows keratinization in most cases. Other types, including adenocarcinoma, estimated as present in 10 per cent by Moore,[1] and an occasional embryonal carcinoma (Ewing[2]) are reported. Adenoacanthoma of the esophagus has received little attention. Kaufmann[3] mentioned that mucus was produced in secondary hepatic deposits of an acanthoma of the esophagus. Ewing[2] stated that the structure of carcinoma of the esophagus may vary in different portions of the tumor, with that in the nodes being either typical of acanthoma or more embryonal and atypical, but he made no reference to a combination of squamous cell carcinoma and adenocarcinoma. Recently, several observers (Wood,[4] Rabson[5] and Pasternack[6]) in reports of cases of adenoacanthoma of the gastrointestinal tract commented on this type as not unusual at the cardioesophageal junction, but only 1 case (Takagi[7]) of the 12 cases of adenoacanthoma of the stomach collected by Wood[4] could be considered as an instance of adenoacanthoma arising at the cardioesophageal junction.

In a survey of the literature we found reports of only 2 additional cases of adenoacanthoma involving the esophagus.[8] Both were Cabot

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From the Laboratory of Pathology, New England Deaconess Hospital.

This investigation was aided by National Cancer Institute Grant no. 301.

1. Moore, R. A.: Textbook of Pathology, Philadelphia, W. B. Saunders Company, 1944, p. 816.
2. Ewing, J.: Neoplastic Diseases, ed. 3, Philadelphia, W. B. Saunders Company, 1940, pp. 605-606 and 936.
3. Kaufmann, E.: Pathology, Philadelphia, P. Blakiston's Son & Co., 1929, vol. 1, p. 641.
4. Wood, D. A.: Arch. Path. **36**:177, 1943.
5. Rabson, S. M.: Arch. Path. **21**:308, 1936.
6. Pasternack, J. G.: Am. J. Path. **11**:541, 1935.
7. Takagi, C.: Gann **31**:173, 1937.
8. (*a*) Cabot Case 21521, New England J. Med. **213**:1311, 1935. (*b*) Cabot Case 19122, ibid. **208**:652, 1933.

cases. In one [8a] only the esophagus was involved, while in the other [8b] the growth occurred at the cardioesophageal junction but the major portion of the tumor was in the stomach. Detailed descriptions and photographs of the tumor as observed in these 2 cases are not available, but it was said to be chiefly adenocarcinoma with limited areas of cornification.

REPORT OF A CASE

The patient was a 46 year old white man. The symptoms directly associated with the tumor were loss of 35 pounds (16 Kg.) and dysphagia over a period of eight months. In the last two months the patient had been unable to swallow solid food. Esophagoscopic examination revealed a tumor in the lower third of the esophagus. On exploration, metastases were found in the tracheal, aortic and retroperitoneal lymph nodes. A gastrostomy was performed, and the patient died two weeks later as a result of empyema of the right pleural cavity.

The anatomic diagnoses were: postoperative gastrostomy, recent; carcinoma of the esophagus with metastases in the liver, the kidney and lymph nodes (mediastinal, periaortic and celiac); pleural empyema, right; pericardial empyema, early; atelectasis of the right lung; pelvic peritonitis.

The primary tumor was entirely confined to the esophagus, being separated from the esophagocardiac junction by 2 cm. of uninvolved mucosa. It was an annular raised growth with well defined edges involving the esophagus over a portion measuring 10 cm. in length. The growth extended through all layers of the wall but did not infiltrate adjacent structures.

The metastases were not extensive. There were three nodules in the liver, two measuring 5 cm. each in diameter and one measuring 1 cm. in diameter. The nodule in the cortex of the left kidney measured 1 cm. in diameter. The lymph nodes along the trachea above the carina, along the abdominal aorta and around the celiac axis were firm and enlarged, and some of them were matted together.

Histologic examinations were made on multiple sections of both the primary tumor and the metastases involving the liver, lymph nodes and a kidney. The main part of the primary tumor and some areas of the metastases showed typical cornifying squamous cells with frequent pearl formation. The interesting and unexpected features of this tumor were revealed chiefly in the metastases, where a combination of adenocarcinoma, undifferentiated carcinoma and keratinizing carcinoma was observed (fig. 1). The particular pattern of this tumor is most commonly seen in certain tumors of the mouth and nasopharynx (Gates [9]).

In the primary tumor to a limited degree, and in a large part of the metastatic deposits, there were various-sized masses of rounded and polyhedral cells. In some instances these masses were sharply outlined by a peripheral layer of cylindric cells. Intimately intermingled with the cells of rounded or polyhedral shape were columnar cells, lined up in circular fashion to surround definite lumens that contained degenerated cells and nuclear debris (fig. 2). In the same masses of cells one noted at fairly frequent intervals single cells or small groups of cells which showed keratinization and in some areas suggested pearl formation (fig. 1). In some of these cell masses there was little or no evidence of keratinization, particularly in the renal metastatic deposit. In the primary

9. Gates, O.: Personal communication to the authors.

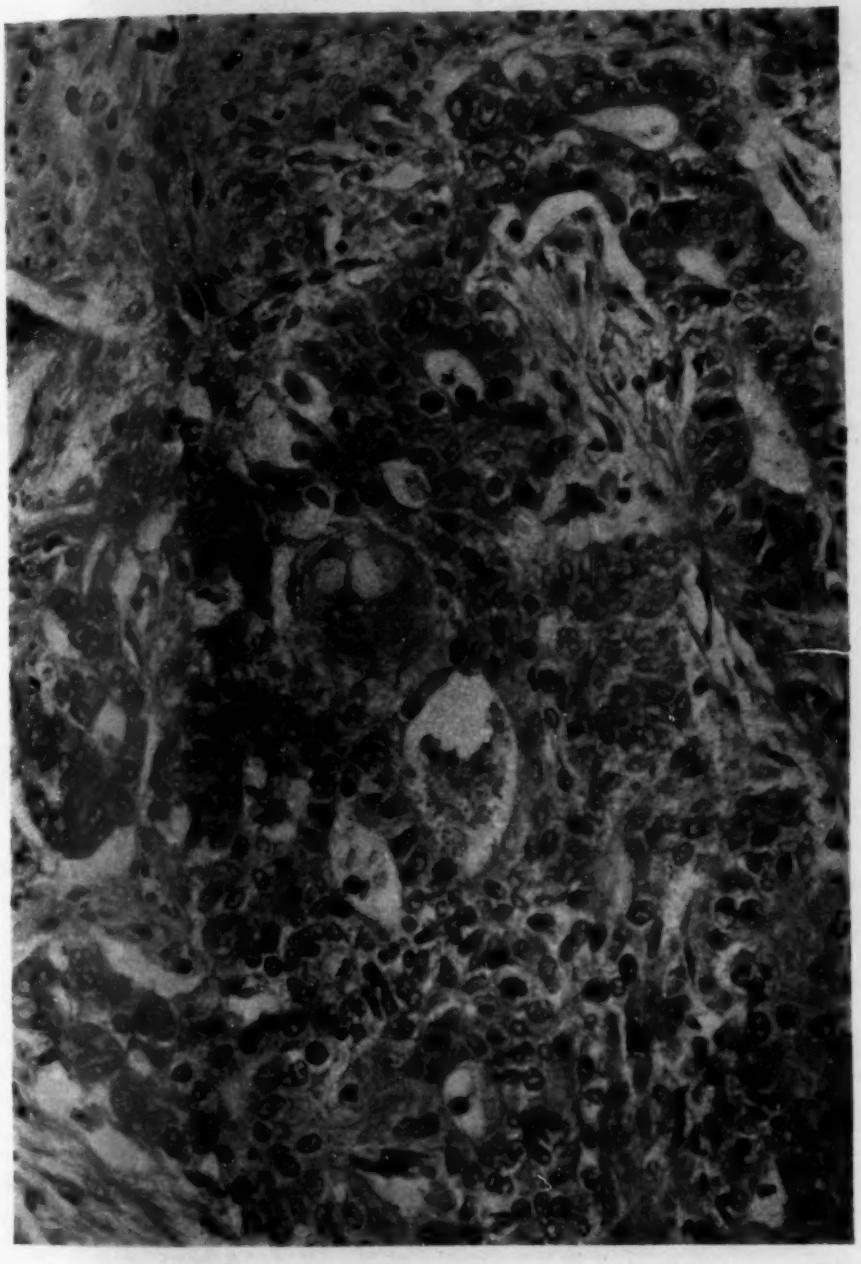

Fig. 1.—Combination of undifferentiated carcinoma and adenocarcinoma in which keratinized cells appear.

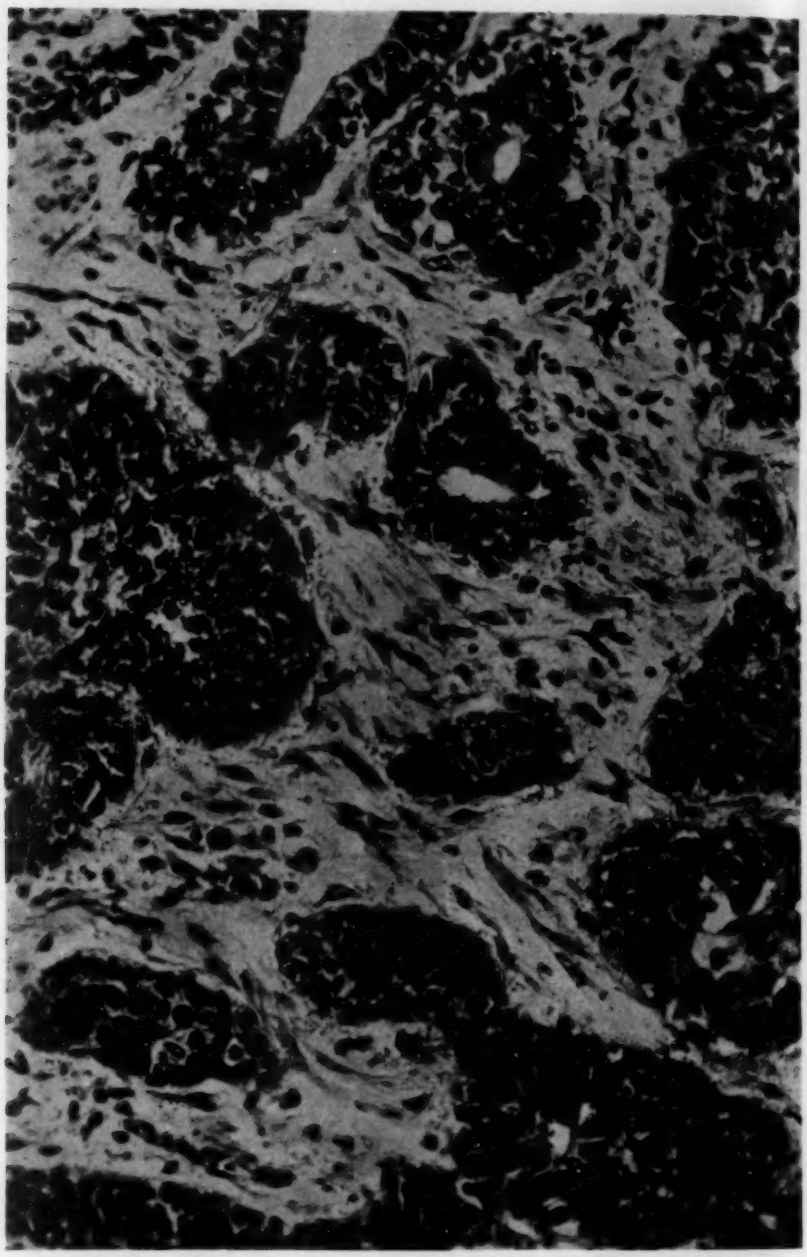

Fig. 2.—Undifferentiated masses of tumor cells with occasional gland formation.

tumor the other extreme was present, where only a few, poorly formed glands were seen near the mucosa of the surface and only a limited number of masses of undifferentiated cells appeared. The borders of the undifferentiated and the cylindric cells were poorly outlined. The eosin-stained cytoplasm of these cells varied from a light acidophilic, finely granular appearance to one which was almost clear. The nuclei were hyperchromatic and relatively large as compared with the cytoplasmic content. They varied only moderately in size or shape as contrasted with the nuclei of the differentiated squamous cells, which displayed marked polymorphism. Nucleoli were present in some nuclei but were not visible in others. Mitoses were extremely rare in the primary tumor but occurred more frequently in the metastatic deposits, up to 2 per high power field. All layers of the esophageal wall were infiltrated by the carcinoma. Necrosis occurred to a marked degree in some portions of the tumor, and central degeneration of the highly cornified carcinoma was common in both the primary tumor and the metastases. The invading tumor evoked a very slight fibroblastic reaction. Inflammatory cellular infiltration was variable, often slight, even in areas of necrosis, but occurred here and there, as evidenced by large groups of lymphocytes, plasma cells and polymorphonuclear and eosinophilic leukocytes.

COMMENT

Our attention was focused on certain special characteristics of this tumor that are usually found in the so-called salivary gland tumors. The tumor was different from the type of adenoacanthoma found elsewhere in the gastrointestinal tract as that usually appears. Comprehensive discussions of the histologic aspects and the histogenesis of that type of adenoacanthoma can be found in the literature.[10]

The importance of the mucous and serous glands of various body cavities as a source of carcinoma has only recently aroused general interest. In 1936 Ahlbom[11] gave a comprehensive account of the recorded tumors of this type, and his careful delineation of tumors of mucous-serous glands of the mouth was the most valuable and instructive contribution on the subject yet made. His work was followed by an equally useful description of similar tumors of the nasal cavities, accessory sinuses and nasopharynx, made by Ringertz[12] in 1938. Other workers, reporting single tumors and groups of tumors of this type, have confirmed and supplemented the observations of Ahlbom and Ringertz. In view of some reports of tumors of mucous and serous glands of the trachea and the bronchi (Ahlbom,[11] Sano and Meade[13]) the current classification of tumors of the lung would seem to need revision. As far as can be ascertained from the literature, no tumor of the mucous and the serous glands of the esophagus has been

10. Scheffler, M. M., and Falk, A. B.: Am. J. Cancer **38**:359, 1940. Ewing.[2] Wood.[4] Pasternack.[6]

11. Ahlbom, H. E.: Acta radiol., 1935, supp. 23.

12. Ringertz, N.: Acta oto-laryng., 1938, supp. 27.

13. Sano, M. E., and Meade, R., Jr.: Arch. Path. **43**:235, 1947.

reported, with exception of a tumor described by Kinoshita [14] which Ahlbom [11] stated had characteristics of the so-called mixed tumor of the parotid gland. In Ahlbom's [11] series there was no mucous or salivary gland tumor of the esophagus, but he stated that the rare reports of adenoma and basal cell carcinoma of the esophagus might relate to tumors belonging to this group. He reminded his readers that the wall of the esophagus contains mucous glands from which such tumors could arise.

In certain of its characteristics the tumor of the esophagus reported in the present paper was identical with portions of some tumors that are described as originating in the so-called salivary type of glands, i.e., mucous and serous glands (Ahlbom [11]; Ringertz [12]; Sheldon [15]; Quattlebaum, Dockerty and Mayo [16]). These characteristics of our tumor are those occasionally found in the areas of undifferentiated and gland-producing carcinoma in which there is keratinization of single or small nests of cells (fig. 1). It is not our intention to classify our specimen definitely with the so-called salivary gland tumors, since we have no proof that it originated from the glands of the esophagus, but it is possible that emphasis on the almost identical histologic patterns of tumors occurring in various locations may be important in the determination of the histogenesis of such tumors.

SUMMARY

A report of 1 case of adenoacanthoma of the esophagus was found in the literature. One additional case of adenoacanthoma of the esophagus is reported. The observation that certain portions of this tumor were similar to the so-called salivary gland tumors is discussed.

14. Kinoshita, M.: Schweiz. med. Wchnschr. **2**:156, 1921.
15. Sheldon, W. H.: Arch. Path. **35**:1, 1943.
16. Quattlebaum, F. W.; Dockerty, M. B., and Mayo, C. W.: Surg., Gynec. & Obst. **82**:342, 1946.

Laboratory Methods and Technical Notes

A MODIFICATION OF MAYER'S MUCICARMINE STAIN FOR MUCIN

MARY DRESBACH
Chief Technician, Section on Pathologic Anatomy, Mayo Clinic
ROCHESTER, MINN.

A MODIFICATION of Mayer's [1] mucicarmine stain for mucin has been employed routinely in staining for mucin in the laboratory of the section on pathologic anatomy of the Mayo Clinic for about two years. During this period more than 1,000 sections of tissues were prepared from current material as well as from stored older tissues which were to be used in special studies. The major advantage of the modification as compared with the original method lies in the marked contrast between the red staining of mucin and the staining of other tissues, which varies from black to blue to green (fig., upper part). With Mayer's original method, the background as well as the mucin tends to assume a pink color. Alcoholic fixation may be used but is not necessary, as most of the sections stained by the method herein reported were fixed in either 4 per cent solution of formaldehyde or Orth's fluid. The technic is rather simple and works well in the hands of technicians who have had little experience.

SOLUTIONS FOR FIXATION AND STAINING

Solutions for Fixation.—A 4 per cent solution of neutral solution of formaldehyde, Orth's solution or dehydrated or 95 per cent alcohol is used.

Solutions for Staining.—1. Weigert's iron-hematoxylin: (*a*) Subsolution A: hematoxylin, 1 Gm.; 95 per cent alcohol, 100 cc. Dissolve with the aid of gentle heat. (*b*) Subsolution B: Iron chloride, 29 per cent aqueous solution, 4 cc.; distilled water, 95 cc.; hydrochloric acid, 1 cc. Mix equal parts of subsolutions A and B. This mixture may be used for several days.

2. Mucicarmine: Carmine, alum lake, 1 Gm.; aluminum chloride, 0.5 Gm.; distilled water, 20 cc. Heat for two minutes or until deep red. Add 80 cc. of 50 per cent alcohol and filter. This solution should be no older than forty-eight hours. Filter again just before use.

3. Indigo Carmine: Two per cent aqueous solution of indigo carmine, 1 cc.; glacial acetic acid, 2 cc.; distilled water, 97 cc. Filter before use.

METHOD

1. Deparaffinize paraffin-embedded sections in the usual manner.

2. Treat tissue for thirty minutes in a freshly prepared solution made by adding

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From the Section on Pathologic Anatomy; J. W. Kernohan and J. E. Edwards, directors of the work.

1. Mayer, P.: Ueber Schleimfärbung [1896], Neapel. Zool. Stat. Mitth. **12**:303, 1897.

glacial acetic acid to saturated aqueous solution of trinitrophenol (picric acid) to the concentration of 5 per cent.

3. Wash in running tap water until the tissue is no longer yellow.

4. Stain with Weigert's iron-hematoxylin stain for three minutes.

5. Remove excess stain by rinsing in tap water.

6. Differentiate in acid alcohol (70 per cent alcohol to which hydrochloric acid has been added to the concentration of 1 per cent).

7. Rinse in tap water.

8. Place in a saturated aqueous solution of lithium carbonate (about 1.3 per cent) for five to ten seconds until the deep blue color is restored.

9. Wash five to ten minutes in running tap water.

10. Place in mucicarmine stain for thirty minutes.

11. Rinse off excess stain in tap water.

12. Counterstain lightly and quickly for about fifteen seconds with the solution of indigo carmine. The slides should be gently agitated while the tissue is being counterstained, so that the stain will be distributed evenly.

13. Rinse in tap water.

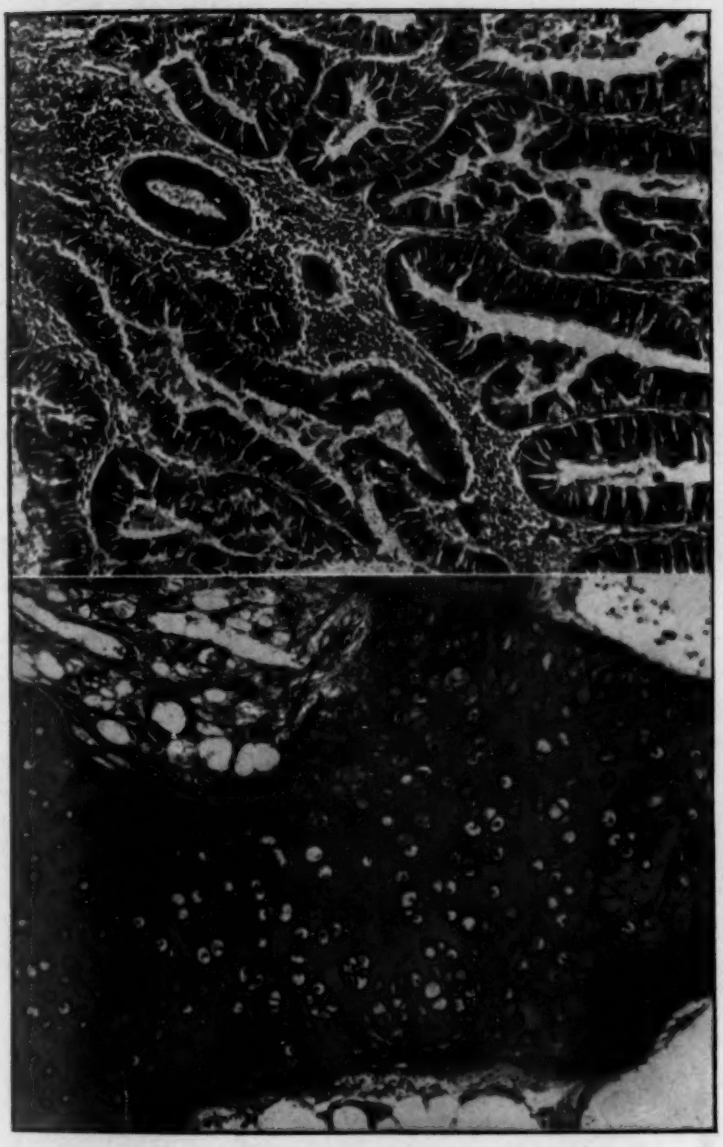
14. Dehydrate in 95 per cent methyl or ethyl alcohol and then in acetone or dehydrated alcohol.

15. Clear in xylene and mount in "clarite" or balsam.

RESULTS AND COMMENTS

Mucus in epithelium and connective tissue stains red; cartilaginous matrix, red; nuclei, black; cytoplasm and collagen, light blue; erythrocytes, green.

The mucus of normal gastric glands stains in a variable manner, as with earlier reported stains. Carcinoma which originates within the gastric mucosa, however, produces mucus which stains positively by this method.



Tissues stained by the method described for mucus; the sites of mucus stain red. Upper part: adenocarcinoma of the rectosigmoid (× 95). Lower part: chondroma of the lung (× 70).

A NEW METHOD FOR BIOPSY OF THE LIVER

GUILLERMO HERRERA, M.D., and VICTORIANO PARDO, M.S.
HABANA, CUBA

THE GREAT INTEREST in hepatic biopsy by puncture [1] prompted us to construct a trocar which makes it possible, with the least amount of trauma and risk to the patient, to obtain sufficient tissue from the liver for a satisfactory histologic study.

The instrument which we present in this paper was constructed after considerable experimenting on cadavers. With it we are able to obtain a cylinder of tissue which enters the cannula after the removal of the stylet. The tissue is cut off by means of a thread at the end of the cannula.

DESCRIPTION OF THE INSTRUMENT

The trocar consists of a cannula with an attached wire and a stylet. The cannula varies from 0.78 to 2 mm. in diameter. At its distal end three holes are present, two smaller ones near the edge and one larger several millimeters farther back (*A* in figure).

The stylet, bevel shaped at its end, protrudes about 2 to 3 mm. beyond the end of the cannula. It is not completely cylindric; one side is flattened out to permit the passing of the two proximal ends of the wire between the cannula and the stylet. As a result the circumference of the stylet forms a segment of a circle. Between the base of this segment and the cannula, space is provided for the wire. The distal end of the stylet is slightly thinner to provide space for the wire loop between the cannula and the stylet (*B* in figure).

A thin wire is used. We found it useful to employ metallic sutures 0.0031 inch (0.0775 mm.) in diameter.[2] The wire should be several inches longer than double the length of the trocar.

The two ends of the wire are introduced through the distal end of the cannula and each passes from the inside outward through one of the two small holes, leaving a loop of several centimeters outside the distal end. Both ends are then passed inward through the larger hole, to run through the entire length of the cannula and emerge at the proximal end. This last step may be facilitated by the use of a heavy wire which serves as a guide.

From the Clinical Service of Prof. C. F. Cardenás, University Hospital, University of Habana.

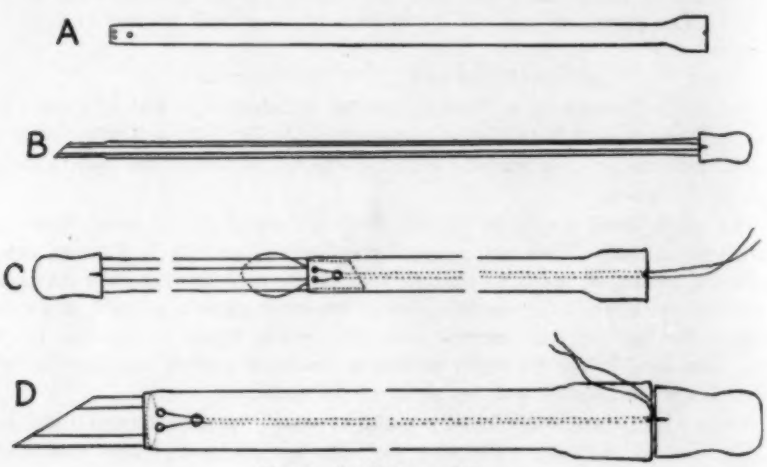
1. Iversen, P., and Roholm, K.: Acta med. Scandinav. **102**:1, 1939. Baron, E.: Arch. Int. Med. **63**:276, 1939. Tripoli, C. J., and Fader, D. E.: Am. J. Clin. Path. **11**:516, 1941. Dible, J H.; McMichael, J., and Sherlock, S. P. V.: Lancet **2**:402, 1943. Hoffbauer, F. W.; Evans, G. T., and Watson, C. J.: M. Clin. North America **29**:363, 1945. Gillman, T., and Gillman, J.: J. A. M. A. **129**: 12, 1945. Gillman, J., and Gillman, T.: Arch. Path. **4**:239, 1945.

2. The wire which we use is marketed under the name Surgaloy, size 40, by Davis & Geck, Inc., 57 Willoughby St., Brooklyn.

The next step is to pass the stylet through the cannula. This is facilitated by holding the wire at tension while the stylet is being passed. When this is accomplished the end of the stylet should pass through the loop of the wire at the distal end. The two free ends of the wire at the proximal end of the cannula are pulled taut. The distal end of the stylet is introduced into the distal opening of the cannula with the wire loop around the stylet (*C* in figure). The two proximal free ends of the wire are pulled taut, then bent forward and held in this position. The stylet is then taken out from the distal end of the cannula and introduced into the proximal end in such a way that the flat side of the stylet runs in the direction of the holes at the distal end of the cannula (*D* in figure).

This part of the procedure is somewhat difficult, but after some practice it can be done in from three to four minutes.

When a trocar is less than a millimeter in diameter the metallic wire may interfere with the tissue entering the cannula. A silk thread may be used instead.



Trocar for biopsy of liver

TECHNIC OF BIOPSY

The portal of entry depends on the condition of the liver. When the liver extends 1 fingerbreadth or more below the right costal margin, we make the puncture through the abdominal wall just below the costal margin, in the midclavicular line. If nodules are felt in the liver, the puncture is made at the level of a nodule.

If the liver is of normal size or slightly enlarged, an area of dulness is demonstrated in the right hypochondrium by percussion. The transpleurodiaphragmatic way is chosen at the level of the seventh or eighth intercostal space in the midclavicular or the anterior axillary line, depending on the size of the area of dulness.

If the liver is small or tilted backward by a distended stomach or intestine, the normal relations between the liver and the anterior part of the diaphragm are disturbed; it is then necessary to use the posterior transpleurodiaphragmatic route; the site of entry is the tenth or eleventh intercostal space about 8 to 10 cm. laterally from the midspinal line.

When the anterior or the posterior transdiaphragmatic route is chosen, the trocar, after it has passed the thoracic wall in the costal interspace, is lowered

until it forms an angle of from 50 to 60 degrees with the wall of the chest, which makes its course perpendicular to the diaphragm. If the liver is small, this course frequently requires piercing the lung where the latter reaches the costodiaphragmatic angle. This has not led to any complications, and piercing the lung is considered preferable to entering below the level of the liver.

The patient is prepared by oral administration of 0.1 to 0.2 grain of sodium seconal. Morphine is not used.

After surgical preparation, anesthesia of the skin and the abdominal wall or of the intercostal space, as the case may be, is obtained by local infiltration. Then a small incision, 3 to 4 mm. long, is made through the skin. The trocar is introduced until it enters the liver. The stylet is then withdrawn and the cannula pushed for a distance of 2 to 3 cm. This will cause a block of liver tissue to enter the cannula as the latter penetrates the liver. The cannula is then held firmly in its position and the two free ends of the wire are pulled. In this manner the base of the cylinder of hepatic tissue is severed. The cannula is pulled out with the biopsy specimen in it. The wire is removed and the block of tissue is pushed out by the stylet. Still better is it to wash out the tissue with the help of a stream of fixative from a syringe.

COMMENT

Our experience of cadavers and of patients prompts us to claim that our method offers several advantages. The trocar causes little trauma, because of its small size and the rapidity of the procedure, thus reducing chances that the liver may be torn by respiratory movements of the diaphragm. We have always obtained sufficient tissue. The relatively long cylinder of tissue gives material from several levels.

We have used the method for biopsies of other organs. It may be of value for the diagnosis of pulmonary neoplasms, though our experience of this type of lesion has been rather limited, and for the study of pneumonia. It is impossible to obtain a biopsy specimen of normal pulmonary tissue or even of tissue slightly consolidated, owing to elasticity. The lung firmly consolidated with pneumonia or tumor can always be easily punched. By obtaining specimens from different levels the trocar enables one to do a true selective biopsy.

We have also used the method on lymph nodes and on tumors of bone and of the breast, with good results.

SUMMARY

A method of obtaining liver for biopsy is described which makes it possible to obtain a cylinder of tissue as long as the distance covered by the trocar in the liver and of the same thickness as the diameter of the trocar. A wire loop at the end of the trocar is an essential feature of the instrument.

The trauma is minimal; abundant tissue is obtained in every case.

We have obtained biopsy specimens from 72 patients without a single hemorrhage or other complication.

STAINING TECHNIC FOR DIAGNOSTIC TISSUE SPREADS

HERMAN J. DICK, M.D.
SYRACUSE, N. Y.

A METHOD adapted from the staining of fecal smears [1] is described which is of use in staining material obtained in aspiration biopsy, body fluids and body secretions.[2] It was found to be superior to other methods, avoiding both the loss of cytologic detail coincident to drying [2d] and the usually unfamiliar color characteristics of the Papanicolaou stains.[3] It provides permanent preparations,[4] and it is offered as an adjunct to, but not as a substitute for, diagnosis by routine paraffin section.[2a,b]

Staining by the following method results in cytologic characteristics which are familiar to most pathologists and has proved satisfactory in most types of smear examination.

1. Fix spreads of fluid or other aspirated material in Schaudinn's solution for two to ten minutes at room temperature. Precise nuclear characteristics are lost if the smear is allowed to dry.[1]
2. Dehydrate in 70 per cent alcohol for five minutes.
3. Remove mercury in 70 per cent alcohol to which enough iodine has been added to give a port wine color—five minutes.
4. Rinse in 70 per cent alcohol.
5. Hydrate through 50 per cent alcohol and water.
6. Stain in Harris' hematoxylin for thirty seconds.
7. Rinse in tap water.
8. Blue in an alkaline solution (a pinch of sodium or lithium bicarbonate added to a staining jar of tap water) for one to two minutes.
9. Stain with 0.1 per cent eosin for two minutes.
10. Dehydrate in graded alcohol, clear in xylene and mount in balsam.

The entire procedure may be done in fifteen to twenty minutes. Spreading with a second slide as in making blood spreads is a convenient method, and it has been noted that individual cells are more easily identified in thin spreads. Spreads may be kept in the fixing solution or in 70 per cent alcohol for forty-eight hours without any harmful results. The fixative may be used until it becomes cloudy. It is impor-

From the Department of Pathology, Syracuse University College of Medicine.

1. Craig, C. F.: Laboratory Diagnosis of Protozoan Diseases, Philadelphia, Lea & Febiger, 1942.

2. (*a*) Foot, N. C.: Am. J. Path. **13**:1, 1937. (*b*) Graham, G. S.: ibid. **9**:701, 1933. (*c*) Herbut, P. A., and Clerf, L. H.: J. A. M. A. **130**:1006, 1946. (*d*) Stewart, F. W.: Am. J. Path. **9**:801, 1933.

3. (*a*) Gates, O., and Warren, S.: Am. J. Path. **21**:567, 1945. (*b*) Shorr, E.: Science **94**:545, 1941. (*c*) Herbut and Clerf.[2c]

4. Altgauzen, A. Y.: Am. Rev. Soviet Med. **4**:148, 1946.

tant that the spreads are not allowed to dry at any point of the procedure.[1] If drying occurs, it is then necessary to depend on size, variation and depth of diffuse coloring of nuclear material [2d] rather than on the sharp nuclear pattern with which pathologists have been familiar in the study of ordinary microscopic sections. Any protein present in the fluid examined is immediately precipitated. This does not interfere with the microscopic examination. Various modifications of the iron-hematoxylin stain and the phosphotungstic acid–hematoxylin stain [1] were not attempted since they are time consuming.

Since the interest was primarily in diagnosing cancer from spreads, nuclear detail [5] was considered most important. The chromosome pattern and the nucleoli retain the conformation and the color characteristics usually seen in material fixed in Zenker's solution. The cytoplasm is stained the usual pink with eosin, and basophilism of the cytoplasm when present is similar to that of good paraffin sections. The introduction of a new galaxy of colors, as in the various Papanicolaou modifications, is avoided.[6] The purpose of these various colors is apparently to permit determination of the origin of the cells or of their functional state. This is desirable for study of cyclic changes but unnecessary in the diagnosis of cancer. Cells as seen in spreads fixed and stained by this method have the same size as cells seen in sections. Individual cells or groups of cells can be identified as easily as in sections. The number of unidentifiable cells is kept to a minimum.

This method is best adapted to aspirated secretions [2] and exudates which are delivered immediately to the laboratory in a fluid state or are spread and fixed immediately at the time of biopsy. It is also well adapted to spreads or impressions of lymph nodes. Smears of peripheral blood and bone marrow are satisfactory in nuclear and cytoplasmic detail except that platelets are not stained. Polychromatic cytoplasm, granules and nucleoli are easily identified. It has been used with good success on material aspirated bronchoscopically from upper lobe bronchi.[2c]

SUMMARY

A more successful method is presented for the fixation and staining of spreads of fluids and exudates. This method emphasizes nuclear detail for identification of cells and minimizes the personal factor of "impression" in the study of smears or tissue fluid.

NOTE.—After this article was accepted for publication, references to a similar technic applied particularly to material aspirated from the bronchi were found in an article by L. S. Dudgeon and C. H. Wrigley (*J. Laryng. & Otol.* **50**;752, 1935) and in one by J. Bamforth (*Thorax* 1:118, 1946). My results are exactly the same as those described and illustrated by Dudgeon and Wrigley.

In view of this there can be no question of priority, the principal results of my experience being (1) the superior fixation of cells in fluids and (2) the advantages of the familiar stains.

5. Altgauzen.[4] Gates and Warren.[3a] Graham.[2b] Herbut and Clerf.[2c]

6. Herbut and Clerf.[2c] Shorr.[3b]

General Reviews

MECHANISMS OF ABNORMAL DEVELOPMENT

I. Causes of Abnormal Development in the Embryo

PETER GRUENWALD, M.D.
NEW YORK

IN RECENT years investigation of abnormal development has gone far beyond the stage in which it consisted mainly of accumulations of descriptions of malformations and speculations as to the probable developmental stages of the anomalies under consideration. It is true that experiments were made long ago with the aim of producing malformations by means of various chemical and physical factors, but the agents were chosen arbitrarily and little of general teratologic significance was gained by this work. Only recent advances in developmental physiology and in genetics, as well as combined work in both fields, have yielded substantial and systematic knowledge of the mechanisms of abnormal development. These will be briefly discussed, and the morphology of malformations will be referred to only as far as is necessary in the course of this discussion of developmental mechanisms.

Many authors have in the past discussed the difficulties of defining malformations. Recent work has increased these difficulties, for it is now known that conditions which are generally recognized as malformations may be produced by a variety of agents which also cause diseases in postnatal life. It is certain that malformations are the result of abnormal development. Since any change of form is development, all abnormal forms might be considered as malformations. There is, for instance, no essential difference between disturbances of early embryonic development caused by chemical agents and, for instance, a tumor forming in the adult after treatment with a carcinogenic chemical. It is important to realize that teratology is a part of pathology not essentially different from the rest.

From the Department of Pathology, Long Island College of Medicine, Brooklyn.

This work was begun while the author was Fellow in Pathology at the Mount Sinai Hospital, New York.

The review of the literature was concluded in August 1946. However, many European journals of the past few years were not available at that time, on account of the interruption of communications during the war.

Knowledge gained in the study of developmental pathology of early stages may offer fundamental points of view for the study of the pathology of the adult, and vice versa. The age at which a disturbance takes place has often been considered as a criterion for malformation, and birth is set as the borderline. This cannot be strictly subscribed to, for identical malformations of teeth, for instance, may develop before and after birth, and typical inflammatory diseases which nobody would regard as malformations have been found in the embryo. No strict definition of a borderline between malformation and disease will be given here, not only because this is impossible but also because it is more profitable to stress the similarities than the differences between the two.

Several authors have discussed broad aspects of teratology in connection with their own descriptive, experimental-embryologic and genetic studies.[1] These reports are good examples of the scope and the success of modern teratology.

There are excellent recent accounts of the sciences which form the basis of developmental pathology. In the field of embryology, particularly in its physiologic aspects, the books of Spemann,[2] Weiss[3] and Needham[4] may be consulted. The last-mentioned author has gathered an impressive amount of the most modern information, including, among others, such borderline fields as embryonic metabolism

1. (*a*) Stockard, C. R.: Am. J. Anat. **28**:116, 1921; (*b*) Am. Naturalist **58**:24, 1924. (*c*) von Szily, A.: Ztschr. f. Anat. u. Entwcklngsgesch. **74**:1, 1924. (*d*) Mohr, O. L.: Ztschr. f. indukt. Abstamm. u. Vererbgsl. **41**:59, 1926; (*e*) Heredity and Disease, New York, W. W. Norton & Company, Inc., 1934. (*f*) Mangold, O.: Ergebn. d. Biol. **7**:193, 1931. (*g*) Wright, S., in Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor, L. I., New York, The Biological Laboratory, 1934, vol. 2, p. 137; (*h*) Physiol. Rev. **21**:487, 1941. (*i*) Mann, I. C.: Developmental Anomalies of the Eye, London, Cambridge University Press, 1937. (*j*) Landauer, W.: Proc. Seventh Internat. Genet. Cong. Edinburgh, 1939; (*k*) Bulletin 236, Storrs Agricultural Experiment Station, 1941. (*l*) Murphy, D. P.: Congenital Malformations, Philadelphia, The Author, 1939. (*m*) Dunn, L. C., in Harvey Lectures, Baltimore, Williams & Wilkins Company, 1940, vol. 35, p. 135; (*n*) Growth (supp.) **5**:147, 1941. (*o*) Potter, E. L., and Adair, F. L.: Fetal and Neonatal Death, Chicago, University of Chicago Press, 1940. (*p*) Snell, G. D.: Biology of the Laboratory Mouse, Philadelphia, The Blakiston Company, 1941. (*q*) Hamburger, V.: Biol. Symposia **6**:311, 1942. (*r*) Grüneberg, H.: The Genetics of the Mouse, London, Cambridge University Press, 1943. (*s*) Gruenwald, P.: Am. J. Anat. **74**:217, 1944.

2. Spemann, H.: Embryonic Development and Induction, New Haven, Yale University Press, 1938.

3. Weiss, P.: Principles of Development, New York, Henry Holt & Company, Inc., 1939.

4. Needham, J.: Biochemistry and Morphogenesis, London, Cambridge University Press, 1942.

and the cancer problem. In the field of genetics, there is, in addition to the standard texts, Grüneberg's [1r] book on the genetics of the mouse, which contains much of the best founded teratologic information now at hand.

The following example will be given to illustrate the changes that have been made in the approach to teratology during the past few decades. Cyclopia, one of the best studied single problems of this field, was first examined with regard to its morphologic aspects, and a series of degrees of the defect was established. It became apparent that the obvious ocular changes are but a part of a more extensive and complex defect, and it was logical to include lesser degrees of the malformation, in which the eyes were not quite fused (synophthalmia). The next step was to trace back the defects of the eyes and the brain to the stage of the flat, open neural plate. By a process comparable to showing a motion picture backward, the tissues of various parts of the eye and the brain were followed back to their position in the neural plate (to a stage in which they are not yet differentiated), and in this map of prospective retina, optic nerve, chiasma and other structures the cyclopic defects were outlined. It was thus shown that the missing structures are comprised in a wedge-shaped symmetric area.[5] The conclusion was reached that a wedge-shaped defect of the early embryo comprising as its most important part portions of the neural plate but also some of the underlying tissue causes cyclopia. Nothing could be said of the nature of this defect beyond the assumption that it never is an open cleft, as if tissue had been cut out, but is rather an absence of parts such that the bordering eye-forming tissue of the two sides develops as one fused mass from the very beginning. By this time experiments had been performed in which cyclopia was produced by treating embryos of various classes of vertebrates with chemicals.[6] The treatment was applied to the whole embryo at early stages, and the mechanism of action was not understood. Destruction of tissue in the aforementioned wedge-shaped area by mechanical means,[7] radiation [8] or electrocautery [8a] was shown to produce cyclopia, but it soon became clear that this was not the mechanism of spontaneous or of chemically produced cyclopia. A new con-

5. Fischel, A.: Arch. f. Entwcklngsmechn. d. Organ. **49**:383, 1921.

6. (*a*) Stockard, C. R.: J. Exper. Zool. **4**:165, 1907; (*b*) Arch. f. Entwcklngsmechn. d. Organ. **23**:249, 1907; (*c*) Anat. Rec. **3**:167, 1909. (*d*) McClendon, J. F.: Am. J. Physiol. **29**:289, 1912. (*e*) Werber, E. F.: J. Exper. Zool. **21**:485, 1916. (*f*) LePlat, G.: Arch. de biol. **30**:231, 1919.

7. Lewis, W. H.: Anat. Rec. **3**:175, 1909.

8. Wolff, E.: Arch. d'anat., d'histol. et d'embryol. (*a*) **18**:145, 1934; (*b*) **18**:229, 1934; (*c*) **22**:1, 1936.

cept stems from experiments made in amphibians. It had long been known that in the early embryo the mesoderm of the notochord and nearby areas acts on the covering ectoderm and induces it to form the neural plate. Adelmann [9] and Mangold [10] were the first to show that median defects produced by excision in the mesoderm of the head caused, apparently also by way of induction, a change in the determinations within the neural plate which had been present in normal size and shape at the time of operation. This neural plate, without being directly affected by the experiment, as shown by controls, formed a cyclopic brain and eye. Thus, not the absence of tissue but abnormal determinations of its prospective parts caused cyclopia. Since induction and thus determination of one part by an adjacent tissue (in this case the mesoderm) are known to be mediated by chemicals released by the latter, one can understand that artificially introduced chemicals may interfere with this process and thus cause cyclopia and in a similar manner other malformations.

Several examples of hereditary median defects, including cyclopia, have been found.[11] This is just one of many instances in which similar if not identical malformations result from hereditary and environmental influences. An inkling of the direction in which the solution of this parallelism is to be sought is given by the increasing volume of evidence that genes act by determining the presence of enzymes and that mutations primarily affect the course of metabolism.[12] Thus the primitive idea of elimination of parts evidently missing in cyclopia has been supplanted by a physiologic concept which is far from complete, but stimulates progress. The formation of cyclopia will be discussed in more detail in part II of this review.

As was indicated by the introductory remarks and the preceding example, the present review will deal with the causes of abnormal development and with the mechanisms which follow the causes and produce the abnormal conditions commonly seen. It has already been stressed that there is no natural borderline between malformation and morbid disturbance of structure. The arbitrary limitation to embryonic disturbances which many writers have imposed on teratology is justified only so far as the principal structural pattern of the organism is determined and produced in the embryo. It is obvious that the most striking and far reaching structural abnormalities must develop in

9. Adelmann, H. B.: J. Exper. Zool. **57**:223, 1930.

10. Footnote deleted by the author.

11. Wright, S., and Eaton, O. N.: J. Agric. Research **26**:161, 1923. Wright, S.: Genetics **19**:471, 1934. Wright, S., and Wagner, K.: Am. J. Anat. **54**:383, 1934.

12. Beadle, J. W., in Harvey Lectures, Lancaster, Science Press, 1945, vol. 40, p. 179; Am. Scientist **34**:31, 1946.

these early stages. For this technical reason (and it should be realized that there is no other) the greater part of this review will also deal with the embryo. Since the close relationship of fetal diseases, such as infection, with malformations is becoming increasingly evident, fetal diseases will also be discussed. In all instances attention will be directed toward representative examples rather than complete listings of references.

The present review will appear in three parts. The first will deal with the causes of abnormal development, the second with the developmental mechanisms of embryonic malformations and the third with examples of conditions in postnatal life which have been adequately analyzed along similar lines, including a brief discussion of the developmental aspects of tumors. Since the causes of most spontaneous developmental aberrations are not known, the second and third parts will have to treat of many conditions which have as yet not been traced to any of the causes described in the first part.

THE CAUSES OF ABNORMAL DEVELOPMENT

If one were to be exact in defining the cause of any particular malformation, it would be necessary to trace the abnormality back to the point where an external force produced the first aberration of any kind from the normal condition. In the case of a hereditary abnormality this means going back through all generations carrying the abnormal gene, to find the external cause of the mutation. This is obviously impossible with the so-called spontaneous mutations, even though one may assume that they, just as those induced in the laboratory, have external causes and are therefore not truly spontaneous in the last analysis. Even in the case of a nonhereditary malformation, in which the cause must have acted on the individual itself, investigators are usually unable to find that cause later on unless they have produced the malformation in the laboratory. This illustrates the value of the experimental production of developmental disturbances, as they are the only ones in which the mechanism can be discovered with any degree of dependability. They are models which show how similar malformations of unknown causes may have developed. However, greatest caution must be observed in applying experimental results to malformations found in nature. The previously quoted example of cyclopia shows that many different causes and even entirely different developmental mechanisms may produce very similar end results. Many more examples of this will be quoted in the following pages.

The causes of abnormal development to be discussed here may be classified as follows:

Genetic causes (including influences on the germ cells probably affecting the genotype):

- Mutations with unknown causes ("spontaneous")
- Mutations induced by { radiation / chemical treatment }
- Hybridization
- Overripeness of the egg cell
- Somatic mutations

Agents affecting the phenotype without effect on the genotype:

- Mechanical agents
- Radiations
- Chemical influences { addition of substances / deficiencies }
- Temperature changes
- Infections

The spatial relation of the causative agent to the affected part has been discussed by Gruenwald.[18] Three possibilities exist for the location of the agent: It may be (*a*) within the primarily affected part, (*b*) within the organism but outside the primarily affected part or (*c*) outside the organism.

The first-mentioned relationship prevails in genetically determined malformations and includes also normal properties of the part in question, which make it more susceptible to extrinsic influences. The second possibility exists if abnormal, or rarely normal, conditions elsewhere in the body cause or favor maldevelopment. The third possibility includes the chemical, physical and infectious agents to be discussed in later sections. It should be noted that this classification includes, in addition to frank teratogenic agents, conditions which are not harmful in themselves but which predispose a given part to abnormal development. No sharp distinction of these two types of agents or conditions is possible. In some cases each is ineffective without the other.[18]

GENETIC CAUSES OF MALDEVELOPMENT

In the case of a typical hereditary malformation an abnormal gene appears by mutation with or without an apparent cause. This gene, in homozygous or heterozygous[13] condition, alone or in combination with other genes or with extrinsic agents, causes abnormal development. The long history of attempts to modify the genetic constitution

13. In the homozygous condition the corresponding places in the two sets of chromosomes of each body cell are occupied by genes of the same quality; in the heterozygous state these two places are occupied by alleles which affect the same traits in different manners, and of which either the normal or the abnormal one may dominate over the other.

will not be reviewed. The problem of the inheritance of acquired properties is still unsolved, as is the related problem of the manner in which species are modified in nature and new species formed. There is, however, unquestionable evidence of the existence of agents by which mutations and consecutive hereditary malformations can be produced. It is generally acknowledged that the genes are located in the chromosomes, which undergo regular and complicated changes during mitotic division of the cell. It is therefore not surprising that agents which are known to have a profound influence on mitosis, namely, certain radiations,[14] are also most potent in the experimental production of mutations. Ultraviolet and roentgen rays have been used extensively in the study of mutations of such primitive organisms as bacteria and fungi,[15] and much of the recent fundamental knowledge of the manner in which genes act on metabolism is derived from this work.

An interesting group of investigations made in amphibians will be mentioned here because the effect is transmitted to the embryo by one of the germ cells, even though it is not certain that the effect is of a genetic nature. Beginning many years ago, numerous workers[16] have irradiated male or female germ cells previous to fertilization and have reported early death of the embryo or, if suitable doses were used, various severe malformations. Among these are abnormalities of gastrulation and defects of the brain and eyes. It was at first surprising that severe irradiation of spermia is followed by normal development. This happens because the chromatin of the spermia is so severely damaged that it does not take part in further development. However, the spermia are still motile and stimulate the egg cells to develop, in a parthenogenetic manner as far as the chromatin is concerned. Accordingly, the embryo has the haploid number of chromosomes, derived entirely from the egg cell.[17] Henshaw[18] confirmed these results and found, in addition to deformities of organs, anaplastic papillomatous growth of the ectoderm, similar to that resulting from development of overripe eggs (see page 407) and other experimental procedures.

The possibility of producing mutations in mammals by the action of roentgen rays has been examined in great detail. Irradiated male mice

14. Politzer, G., in Chambers, R., and others: Protoplasma-Monographien, Berlin, Verlagsbuchhandlung Gebrüder Borntraeger, 1934, vol. 7.

15. Gray, C. H., and Tatum, E. L.: Proc. Nat. Acad. Sc. **30**:404, 1944. Beadle.[12]

16. (*a*) McGregor, J. H.: Science **27**:445, 1908. (*b*) Bardeen, C. R.: Am. J. Anat. **11**:419, 1911. (*c*) Hertwig, O.: Arch. f. mikr. Anat. **77**:1, 1911; (*d*) **77**:165, 1911; (*e*) (supp.) Anat. Anz. **54**:94, 1921. (*f*) Rugh, R.: Proc. Am. Philos. Soc. **81**:447, 1939.

17. Hertwig.[16e] Rugh.[16f]

18. Henshaw, P. S.: J. Nat. Cancer Inst. **3**:409, 1943.

had offspring with hereditary malformations of brain, eyes, face, extremities and urogenital tract. These abnormalities have been followed through many generations.[19a] Their embryologic aspects will be described in part II. The causal relationship between irradiation and the origin of this mutation has recently been questioned,[19r] but there are other instances, involving mostly malformations of the brain, in which the hypothesis that the mutation is caused by roentgen rays is more probably true.[20]

This work has raised the question whether or not therapeutic irradiation of the ovaries or the testes of man can be the cause of abnormal offspring by producing mutations. This has been discussed extensively by geneticists as well as by clinicians. It is obvious that damage to the offspring by mutations induced in the parental germ cells must be clearly distinguished from noninherited damage due to the embryos having been irradiated in utero. The former may not be apparent until two or more generations hence. The latter, which is definitely known to produce malformations, will be referred to in a subsequent section.

There is no direct proof that irradiation of the maternal ovaries preceding conception produces malformations in the offspring. A review of 265 cases [21] showed 5 per cent defective children, but it was pointed out that these small numbers of defects not conforming to any definite pattern of maldevelopment may well be due to other influences of the environment of the fetus, since the mothers had some pathologic conditions requiring roentgen therapy. Another writer [22] asserts with questionable logic that there is no danger for pregnancies following therapeutic irradiation, even though he has found an increased incidence of abortions as well as of retarded development of the children in later years. The possibility of roentgen ray damage of the internal genital organs of the mother with the result that the development of the ovum may be interfered with must also be taken into consideration.[23] Several authors have reviewed animal experiments, some of which were referred to in foregoing paragraphs, and have concluded that damage of the genetic constitution of the immature egg cells of the ovary is improbable if not impossible.[24] Statistical evaluation of the probability of mutations led to similar conclusions.[25] However, several authors

19. Little, C. C., and Bagg, H. J.: J. Exper. Zool. **41**:45, 1924. Little, C. C.: Am. Naturalist **65**:370, 1931.

20. (*a*) Snell, G. D.; Bodemann, E., and Hollander, W.: J. Exper. Zool. **67**:93, 1934. (*b*) Snell, G. D., and Picken, D. I.: J. Genet. **31**:213, 1935. (*c*) Snell, G. D.: Radiology **36**:189, 1941.

21. Murphy, D. P.: Surg., Gynec. & Obst. **48**:766, 1929.

22. Werner, P.: München. med. Wchnschr. **68**:767, 1921.

23. Borak, J.: Arch. f. Gynäk. **147**:304, 1931.

24. Murphy, D. P.: Surg., Gynec. & Obst. **47**:201, 1928. Borak.[23]

25. Peller, S.: Arch. f. Gynäk. **147**:360, 1931.

recommend caution and protection of the gonads from unnecessary irradiation even though the probability of ill effects may be small.[26] Schubert [26d] points out in this connection that in the production of mutations only the total dose counts, regardless of the size and intervals of the single doses, whereas Müller [26b] gives a maximum daily dose which he considers safe. No objection to diagnostic doses as used for roentgenograms has so far been raised. Snell [26c] holds that while gene mutations (affecting single genes) are not to be expected, chromosome mutations, such as translocation of larger portions of a chromosome, are far more probable. He agrees with other writers that the only proved instance of damage of the offspring due to irradiation of the germ cells is that of damage caused by treatment of mature sperm cells. It thus appears to be the opinion of most authors that while maldevelopment has been proved to occur only when mature sperm cells have been irradiated shortly before fertilization, greatest caution should be exerted and the gonads of persons in the reproductive age protected from roentgen rays. In the male the typical irradiation effect consists of a transient period of sterility, which does not immediately follow the treatment. Only fertilization occurring during the period between treatment and this temporary sterility is definitely known to produce defective offspring.[27]

Little conclusive work has been reported on hereditary changes produced by methods other than irradiation. There are a few reports on the offspring of animals treated with chemical agents. They are important from the medical point of view because they concern substances to which human beings may be exposed. Stockard and his coworkers [28] describe in the descendants of alcoholized guinea-pigs a hereditary inferiority resulting in a reduced number of offspring and early death of many young. Pearl [29] exposed chickens to vapors of ethyl alcohol, methyl alcohol or ether and found a reduction in the number of fertile eggs but a lower prenatal and postnatal mortality of the chickens derived from fertile eggs as compared with his controls. There was also a higher mean adult body weight, and no increase in the incidence of malformations. In order to explain the discrepancy of his own results and those of Stockard and others not mentioned

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26. (*a*) Murphy, D. P., and Goldstein, L.: Am. J. Roentgenol. **22**:207, 1929. (*b*) Müller, J. H.: Monatschr. f. Geburtsh. u. Gynäk. **109**:105, 1939. (*c*) Muller, H. J.: Nature, London **147**:718, 1941. (*d*) Schubert, G.: Röntgenpraxis **13**:1, 1941.

27. Borak.[28] Snell.[26c]

28. Stockard, C. R., and Craig, D. M.: Arch. f. Entwcklngsmechn. d. Organ. **35**:569, 1912. Stockard, C. R., and Papanicolaou, G. H.: Am. Naturalist **50**:144, 1916.

29. Pearl, R.: J. Exper. Zool. **22**:241, 1917.

here, Pearl proposes the following hypothesis: "The germ cells of a given species differ in their capacity to produce normal, sturdy offspring and also in their resistance to damage caused by alcohol or other means." These two properties are coupled so that in some species and under certain conditions alcohol will kill those cells which would otherwise produce weak offspring and leave the others unharmed, and in other species alcohol will also damage the remaining cells. The former would explain Pearl's own results with chickens, and the latter, Stockard's findings in guinea pigs. In this manner the seemingly conflicting results may be explained. In a more recent review of the subject, P. Hertwig [30] cites many articles accepting or denying an effect of alcohol on the progeny, and concludes that the final answer has yet to be found.

Landauer [31] published a preliminary report on the offspring of cocks treated with thallium. There was a high mortality during a narrowly limited period of time within the first two weeks after hatching, which varied slightly with the intensity of the fathers' treatment. These experiments have not been continued on a satisfactory scale, and Landauer himself [32] considers his results as significant but not conclusive.

The offspring of guinea pigs affected by lead poisoning have been examined.[33] They showed a reduced birth weight, an increased death rate during the first postnatal week and general retardation of development. When the lead treatment ends, the gonads recover, and the new progeny is normal. There is no mention of offspring of the retarded young.

Somewhat doubtful is the interpretation of hereditary malformations of the eye, of the progeny of rabbits treated with the serum of fowls immunized to lens tissue.[34] The malformations in later generations include not only opacity of the lens but also microphthalmia with defects of other parts of the eyes. In the progeny of chickens treated with naphthalene or alcohol, cataract and coloboma have been described.[35]

The effect of overripeness of the egg cell on development should be mentioned at this point, even though there is no conclusive evidence that it is due to changes in the genetic structure. However, the effect is transmitted to the embryo by one of the germ cells, just as were the radiation effects in some of the aforementioned experiments on germ cells. Overripeness of the egg at the time of fertilization has been studied in several classes of vertebrates and found to be associated

30. Hertwig, P.: Jahresk. f. ärztl. Fortbild. **26**:50, 1935.

31. Landauer, W.: Arch. f. Gewerbepath. u. Gewerbehyg. **1**:791, 1931.

32. Landauer, W.: Personal communication to the author.

33. Weller, C. V.: J. M. Research **28**:271, 1915.

34. Guyer, M. F., and Smith, E. A.: J. Exper. Zool. **31**:171, 1920. Davis, F. A.: Tr. Ophth. Soc. U. Kingdom (pt. 2) **45**:555, 1925.

35. Kusagawa, S.: Arch. f. Ophth. **118**:401, 1927.

with a reduction in the number of offspring and a variety of developmental disturbances. In the trout various malformations, including double monsters, were found, as well as a change of the sex ratio in favor of males.[36] The latter has been explained by transformation of some genetic females, due to a somatic cause. Similar sex changes in frogs developing from overripe eggs were studied in detail by Witschi.[37] Various malformations, including duplicitas, were also found in them.[38] As a severe form of this disturbance, entirely unorganized growth was observed which, when transplanted to normal tadpoles, grew in the manner of a cancer.[39] Other investigators do not assume a true cancerous nature, pointing out that only in weak hosts aggressive growth occurs.[40] In the guinea pig and the rat a reduced number of embryos, frequent death in utero, and malformations follow delayed insemination.[41] No normal development occurs in the guinea pig if fertilization is delayed more than twenty hours after ovulation, and no development at all if the interval is longer than thirty-two hours. In the rat the limit for normal development of at least part of the embryos is fertilization ten hours after ovulation. In most mammals overripeness of the egg is normally prevented, as the female admits the male only during estrus, i. e., at the optimal time. Nothing is known to suggest the occurrence of malformations due to overripeness in man, in whom no such protective mechanism exists.

Another probable instance of genetically controlled malformations has been reported but not sufficiently worked out. Loeb[42] and Newman[43] found that eggs of the fish Fundulus when fertilized with spermia of other fish species yield large numbers of various malformations. These malformations resemble those Werber obtained by chemical treatment of normally fertilized eggs (page 417) and include, among others, double monsters, cyclopia and various other defects of large parts of the body, leaving in some instances only isolated eyes or hearts. Loeb is inclined to assume that in these cases the eggs develop parthenogenetically as far as their sets of chromosomes are concerned—in other words, that the chromosomes of the spermia are

36. Mršić, W.: Arch. f. mikr. Anat. u. Entwcklngsmechn. **98**:129, 1923.

37. Witschi, E.: Arch. f. Entwcklngsmechn. d. Organ. **102**:168, 1924

38. Witschi, E.: Proc. Soc. Exper. Biol. & Med. **31**:419, 1934. Zimmerman, L., and Rugh, R.: J. Morphol. **68**:329, 1941.

39. Witschi, E.: Proc. Soc. Exper. Biol. & Med. **27**:475, 1930.

40. Briggs, R. W.: Anat. Rec. **81**:121, 1941. Briggs, R. W., and Berrill, N. J.: Growth **5**:273, 1941.

41. Blandau, R., and Young, W.: Am. J. Anat. **64**:303, 1939. Blandau, R. J., and Jordan, E. S.: ibid. **68**:275, 1941.

42. Loeb, J.: J. Morphol. **23**:1, 1912; Biol. Bull. **29**:50, 1915.

43. Newman, H. H.: Biol. Bull. **32**:306, 1917.

lost. In support of this he shows that similar malformations can be obtained from normally fertilized eggs by various external influences. However, it was mentioned in a preceding paragraph that if spermia damaged by various agents [16] or derived from a different species [44] merely induce parthenogenesis, the incidence of malformations is not unusually high. Further investigation of this problem should be of great interest.

Numerous hereditary malformations of unknown cause have been found and extensively studied in the breeding of laboratory and domestic animals. Several of these have been investigated embryologically, with highly gratifying results, as will be reported in part II.

The details of the genetic mechanisms involved will not be discussed here, as such an excursion would lead far into the field of genetics. Several reviews of various aspects of the subject are available.[45] Many of the well studied hereditary malformations are transmitted by single factors, the abnormal trait being either dominant or recessive, or having a different expression in homozygous and heterozygous form. A large number of hereditary traits are known which in the homozygous condition interfere with life beyond embryonic or early postnatal periods, and the genes producing these severe malformations in homozygous individuals are called lethal factors. The embryos carrying this condition and destined to die before reaching maturity should, according to Cairns,[46] be called prothanic rather than lethal. The best studied example is the Creeper chick which will be discussed later in more detail.

The expression of single genetic factors may be modified by other genetic [47] or by environmental [48] factors. Some of these modifications increase the severity of the defect. There is, for examples a cumulation of severity of malformations if Creeper chick embryos develop under the influence of Selenium intoxication.[49] Other instances are known in which the embryo may benefit from the action of additional influences. Remarkable among these are: changed uterine environment in an early lethal malformation of the mouse,[50] temporary lowering of

44. Rugh, R., and Exner, F.: Proc. Am. Philos. Soc. **83**:607, 1940.

45. (*a*) Mohr.[19] (*b*) Snyder, L. H.: Medical Genetics, Durham, N. C., Duke University Press, 1941; (*c*) Am. Naturalist **76**:129, 1942. (*d*) Baur, E.; Fischer, E., and Lenz, F.: Human Heredity, New York, The Macmillan Company, 1931.

46. Cairns, J. M.: J. Exper. Zool. **88**:481, 1941.

47. (*a*) Reed, S. C., and Snell, G. D.: Anat. Rec. **51**:43, 1931. (*b*) Reed, S. C.: Genetics **21**:361, 1936. (*c*) Dunn.[1n] (*d*) Grüneberg.[1r] (*e*) Dunn, L. C., and Gluecksohn-Schoenheimer, S.: Proc. Nat. Acad. Sc. **31**:82, 1945.

48. Dunn, L. C.; Gluecksohn-Schoenheimer, S.; Curtis, M. R., and Dunning, W. F.: J. Hered. **33**:65, 1942. Dunn.[1n] Grüneberg.[1r]

49. Landauer, W.: J. Exper. Zool. **83**:431, 1940.

50. Robertson, G. G.: Genetics **27**:166, 1942.

the incubation temperature [51] and possibly also increased oxygen supply [46] in the case of the homozygous Creeper chick. Similarly, the development of hereditary polydactyly in chickens may be suppressed by lowering the temperature at incubation.[52] However, no influence of lowered temperature was found in several other hereditary malformations of the chick.[53]

Another noteworthy fact is that identical or very similar abnormalities are produced by mutations occurring at different points of the pattern of genes.[54] Finally one should mention the peculiar effect which results if an abnormal genetic factor is located in the sex chromosome. The possibilities for the expression of this factor will then differ according to sex, and this has been termed sex-linked inheritance. Many examples have been described in human and animal pathology—for example, hemophilia and certain forms of color blindness. For the details of this hereditary mechanism textbooks of genetics should be consulted.

The mechanism of gene action as observed during the development of an individual and throughout life is not well understood. In some instances it appears that the presence of enzymes is governed by genes, and genetically controlled enzyme deficiencies are followed by accumulation or excretion of intermediary products which cannot be metabolized. This has been studied in detail in fungi, and similar conditions seem to be present in human alkaptonuria and phenylketonuria,[12] lipid storage diseases [55] and glycogen storage disease.[56] Structural abnormalities are not readily explained by this mechanism. Only in the case of gargoylism (lipochondrodystrophy) it has been suggested that the disturbances in skeletal development are caused by accumulations of an as yet unidentified substance in the cartilage cells.[57] In many malformations the gene action is supposed to affect the rate of metabolism and of growth at a definite stage of development,[4] interfering primarily with those parts which grow most actively at that moment.[18] The problem of multiple ("pleiotropic") gene action will be discussed in part II of this review.

In the preceding pages only those malformations have been considered which are caused by the action of abnormal genes. There

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51. Landauer, W.: Science **100**:553, 1944.

52. Sturkie, P. D.: Genetics **27**:172, 1942; J. Exper. Zool. **93**:325, 1943.

53. Sturkie, P. D.: Am. Naturalist **79**:286, 1945.

54. Dunn, L. C., and Gluecksohn-Schoenheimer, S.: Proc. Nat. Acad. Sc. **30**:173, 1944.

55. Sobotka, H.; Glick, D.; Reiner, M., and Tuchman, L.: Biochem. J. **27**:203, 1933. Sobotka, H.: J. Mt. Sinai Hosp. **9**:795, 1942.

56. Bridge, E. M., and Holt, L. E.: J. Pediat. **27**:299, 1945.

57. Schmidt, M. B.: Centralbl. f. allg. Path. u. path. Anat. **79**:113, 1942.

are, however, a few conditions in which abnormalities develop through unfavorable constellations of genes which are normal in themselves. This is the case in certain instances of genetic intersexuality.[58] While the intersexuality which occurs in some breeds can be accounted for by gene mutations,[59] that in others has been produced by abnormal combinations of normal genes. There are not only polyploid individuals (in lower species of animals) in which abnormal numbers and proportions of normal male and female determining factors can produce abnormal sex development by their interaction, but also diploid individuals in which intersexuality has been found to be due to a faulty quantitative relationship of sex determining factors.[60] This has been studied extensively by Goldschmidt [61] in crosses of various local races of the gypsy moth, Lymantria dispar. It was found that some of these races differ in the quantitative effect of their male and female determining factors. Within each race the strengths of these factors are so matched that a sufficient preponderance of one sex is present to assure normal sex development. In crosses, however, this preponderance may be insufficient if male and female factors come from races with factors of different strength. Thus, in an individual which, according to the number of its sex chromosomes, should be female, the male factors may be relatively too strong, or vice versa, and intersexuality will result. The two opposing views on the manner in which intersexual traits develop in these individuals during embryonic life will be discussed in part II. It must be emphasized that in most species the strengths of the sex factors do not vary among races and, consequently, the overwhelming majority of interracial crosses will not result in intersexuality. There are few examples of intersexuality appearing in interracial crosses in vertebrates, and these have not been fully analyzed from the genetic angle. If this analysis could be undertaken, these instances might well turn out to be due to mutations rather than to an abnormal combination of normal factors as was just discussed.

In another instance of developmental disturbances caused by normal genes the mechanism is entirely different. It is not an interaction of ill matched genes during the development of the traits which they

58. The term "intersexuality" was used by Goldschmidt [61] to indicate those abnormal sexual conditions which result from sex reversal during development (see part II of this review). This theory is not generally accepted. The term is now frequently used to indicate sexual intergrades of various kinds.

59. (*a*) Bonnevie, K.: Arch. f. Entwcklngsmechn. d. Organ. **106**:611, 1925. (*b*) Riddle, O.; Dunham, H. H., and Schooley, J. P.: Genetics **27**:165, 1942. (*c*) Eaton, O. N.: ibid. **30**:51, 1945.

60. Bridges, C. B., in Allen, Danforth and Doisy,[64] p. 15.

61. Goldschmidt, R.: Die sexuellen Zwischenstufen, Berlin, Julius Springer, 1931.

determine, but an effect of incompatibility of these traits themselves after they have developed. This is the case when an embryo of an Rh-positive blood group sensitizes the mother[62] whose blood does not contain Rh factor. As a rule, the embryo develops undisturbed unless the mother has previously been sensitized by Rh-positive substance, usually in the course of an earlier pregnancy, and has developed antibodies. These, transmitted to the embryo through the placenta, produce deleterious effects, the best known of which is the so-called erythroblastosis fetalis. Details of the ill effects of this constellation on the embryo will be described later. Other blood group incompatibilities between mother and fetus account for a small percentage of cases of erythroblastosis.[63]

There is a controversial and possibly highly important genetic cause of localized developmental abnormalities, namely, somatic mutation. This means that during the development of the embryo a mutation arises in one cell and is transmitted only to the descendants of that cell, which are thus genetically different from all other cells of the body. Most striking are those cases in which this appears to have happened at the first division of the fertilized egg cell, and one half of the body differs from the other in some genetically determined character. Examples are gynandromorphism,[63] unilateral gigantism[65] and unilateral pigmentary anomalies.[66] In arthropods genetic mosaics of various kinds are well known.[67] Another field in which somatic mutations have been taken into consideration is that of cancer.[68] This will be referred to in part III of this review.

ENVIRONMENTAL CAUSES OF MALDEVELOPMENT

It is obvious that abnormal development can be induced by any number of physical or chemical agents which will damage but not kill

62. Levine, P.; Burnham, E.; Katzin, E. M., and Vogel, P.: Am. J. Obst. & Gynec. **42**:925, 1941. Levine, P.: J. Pediat. **23**:656, 1943. Davidsohn, I.: J. A. M. A. **127**:633, 1945.

63. Polayes, S. H.: Am. J. Dis. Child. **69**:99, 1945.

64. Allen, E.; Danforth, C. H., and Doisy, E. A.: Sex and Internal Secretions, Baltimore, Williams & Wilkins Company, 1939.

65. (*a*) Mohr.[1e] (*b*) Hollander, W. F.: Quart. Rev. Biol. **19**:285, 1944. (*c*) Warren, D. C.: J. Hered. **36**:227, 1945. (*d*) Wartenberg, R.: Arch. Neurol. & Psychiat. **54**:75, 1945. (*e*) Zondek, L. H.: Arch. Dis. Childhood **20**:35, 1945. (*f*) Rugel, S. J.: Am. J. Dis. Child. **71**:530, 1946.

66. Zlotnokoff, M.: J. Hered. **36**:163, 1945. Glass, B.: ibid. **36**:192, 1945.

67. Goldschmidt.[61] Mohr.[1e]

68. (*a*) Furth, J.; Boon, M. C., and Kaliss, N.: Cancer Research **4**:1, 1944. (*b*) Furth, J., in Luck, J. M.: Annual Review of Physiology, Stanford University, Calif., Annual Reviews, Inc., 1944, vol. 6, p. 25. (*c*) Strong, L. C.: Arch. Path. **39**:232, 1945; Yale J. Biol. & Med. **18**:359, 1946.

the embryo. No attempt will be made here to review the large amount of older work in which various agents were applied to embryos at random and without regard to the mechanism of action. Great caution must be exerted in the evaluation of such work, because unknown accidental factors may be more potent than those intended to act. An example of this is the extensive work of Ferret,[69] who found that opening the shell of the hen's egg or manipulating the albumin of the egg has a profound influence on the embryo. Innumerable reports of experimental work with chick embryos have appeared since, and the necessary manipulations of the shell, of the albumin or even of the embryo itself have not produced the severe changes described by Ferret. On the other hand, workers in this field are often faced with the occurrence of numerous severe malformations in their material without an apparent cause. Shaking the eggs while they are being shipped to the laboratory has often been indicated and is certainly not without effect if it reaches a certain intensity. A few other causative agents have tentatively been identified—for example, fumes of a laboratory [70] or jarring.[71]

Mechanical Agents.—Displacement or destruction of parts of the embryo may be produced mechanically in accidental injuries or in surgical experiments. Comparable destructions are produced when parts of embryos are destroyed by chemical action or radiation, in contrast to true chemical or actinic action on development in which the affected tissue survives and shows the effect of the agent. Thus, electrolysis and roentgen rays have been used extensively in experimental embryology and teratology in order to eliminate certain tissues. The procedure is less hazardous than mechanical excision, and its effect on further development is essentially the same. Many of the common severe malformations, such as cyclopia or that of the sirenomelus, have been reproduced in chick embryos by localized roentgen ray destruction.[8c]

There are other kinds of mechanisms interfering with development in which the action is not so obvious. Numerous reports on invertebrates and lower vertebrates centrifuged early in their development will not be reviewed. A recent analysis of the literature and original work [72] relating to frogs revealed that at the early blastopore stage certrifugation is most effective in the production of malformations, and the optimum speed is about 2,500 revolutions per minute. Higher speeds cause early death. The malformations are varied and include, among

69. Ferret, P. E.: Arch. d'anat. micr. **7**:1, 1904.

70. Stockard, C. R.: Anat. Rec. **8**:33, 1914.

71. Stiles, K. A., and Watterson, R. L.: Anat. Rec. **70**:7, 1937.

72. Torrey, T. W., and Breneman, W. R.: Proc. Indiana Acad. Sc. **50**:213, 1941.

others, cyclopia and other defects of the head region, duplication of the caudal portion of the body and persistent open blastopore. The authors believe that dislocation of a portion of the organization center causes the defects of the head and the duplication of caudal parts.

An investigation of the effect of jarring on chick embryos [71] was precipitated by an unexplained increase in the occurrence of certain severe malformations in a laboratory in which other activities were also carried out. These malformations, including platyneuria (a peculiar form of nonclosure of the neural plate) and absence of yolk sac circulation, causing death, were reproduced by making a weight strike the table with the incubator many times in rapid succession during the early hours of incubation. In another series of experiments mechanical shaking of hen's eggs prior to incubation increased the incidence of all types of malformations, notably of "accidental" rumplessness.[72] In this case a combination of the mechanical factor and a genetic one was revealed, as the incidence of rumplessness was increased particularly in the offspring of those hens which were known to produce occasional rumpless chickens even without shaking.

In man and other mammals intrauterine development reduces the occurrence of mechanically caused malformations to a minimum. In the early phases of teratology, mechanical explanations were favored for almost all malformations and a narrow amnion or amniotic bands and adhesions were commonly indicated as the cause. Today investigators are more critical, and the incidence of these factors is found to be low when one relies on positive criteria, such as the demonstrable presence of amniotic bands and not just uncharacteristic furrows and irregular defects.[74] Moreover, some of the intrauterine "amputations" are known to be inherited in almost identical form,[75] which disproves an amniotic band causation. However, the occasional occurrence of malformations due to narrowing of the amniotic cavity (oligohydramnios) or to constriction of amniotic bands has been demonstrated beyond doubt.[74] That amniotic adhesions may be not accidental but sequelæ of defective conditions of the embryonic tissues, has been suggested by Streeter [76] and Seitz.[77] The histologic changes in the stumps of seemingly amputated legs of an infant have been described in detail by Taylor Gorostiaga and Lede.[78] The effect of constriction of em-

73. Landauer, W., and Baumann, L.: J. Exper. Zool. **93**:51, 1943.

74. Gruber, G. B., in Schwalbe, E., and Gruber, G. B.: Die Morphologie des Missbildungen des Menschen und der Tiere, Jena, Gustav Fischer, 1937, vol. 1, pt. 3, p. 278.

75. Koehler, O.: Ztschr. f. menschl. Vererb.- u. Konstitutionslehre **19**:670, 1936.

76. Streeter, G. L.: Contrib. Embryol. **22**:1, 1930.

77. Seitz, L.: Monatschr. f. Geburtsh. u. Gynäk. **94**:236, 1933.

78. Taylor Gorostiaga, D., and Lede, R. E.: Prensa méd. argent. **31**:363, 1944.

bryonic extremities, with subsequent rapid autolysis, has been investigated experimentally in mammals.[79]

Gross mechanical injury rarely leads to malformations in man and other mammals. If the injurious force penetrates the protecting envelopes, abortion or, in some mammals, resorption is the most probable outcome. In the recorded cases in which human embryo obviously survived an injury, the brain as the most vulnerable part is the most commonly affected. Pertinent examples are (1) apparently hemorrhagic destruction of both hemispheres following an accident during pregnancy [80] and (2) atypical presence of encephaloceles not in the embryonic line of closure of the primordium of the brain, with embolism and growth of brain tissue in the lungs.[81]

Mechanical conditions unfavorable for normal development may prevail in twins. Omphalocephaly [82] and ourentery [83] (anomalies in which the cranial and the caudal end of the body, respectively, grow into the yolk sac) of twins are probably caused by pressure of one twin on the other, in contrast to similar malformations of single embryos, which are probably not caused by external pressure.[84] The development of an acardiac twin may have a hydromechanic cause if one of twins, by virtue of his stronger circulation, takes over the function of propelling blood through the common placenta and the other twin. The latter will then suffer complete degeneration of large parts of his body. This course of events is hypothetic, and it is quite probable that at least in part of the cases the acardius is primarily maldeveloped or has abnormal vascular relations to his co-twin and the placenta which secondarily lead to the changes of circulation just described.[83]

Radiations.—An embryo may be deformed by radiation in two ways. One, in which a defect is caused by destruction of the irradiated tissue, has been mentioned in the preceding section. The other possibility is the survival of damaged tissue. While complete elimination of some tissue can usually not be ruled out, the following reports deal, in all probability, mainly with effects of the latter type. Hinrichs and Gentner [85] produced twins and double monsters in fish eggs with ultraviolet radiation, and determined as the most effective period the time just before the onset of cleavage. Many other malformations were also found, and various organs appeared to be affected to different degrees. The

79. Hellner, H.: Monatschr. f. Geburtsh. u. Gynäk. **95**:70, 1933.

80. Seitz, L.: Arch. f. Gynäk. **83**:701, 1907.

81. Gruenwald, P.: Am. J. Path. **17**:879, 1941. Potter, E. L., and Young, R. L.: Arch. Path. **34**:1009, 1942.

82. Gruenwald, P.: Ztschr. f. Anat. u. Entwcklngsgesch. **107**:782, 1937.

83. Gruenwald, P.: Anat. Rec. **83**:267, 1942.

84. Gruenwald, P.: J. Morphol. **69**:83, 1941.

85. Hinrichs, M. A., and Gentner, I. T.: Physiol. Zoöl. **4**:461, 1931.

circulatory and the central nervous system are more severely affected than other parts. Solberg [86] has reported on fish embryos that were exposed to roentgen rays. He distinguishes the following stages of the effect: (1) a latent period; (2) retardation through interference with mitosis; (3) disintegration of some tissues; (4) reorganization; (5) subsequent differentiation, depending on the changes previously produced. Uniform malformations can be obtained by properly controlled irradiation. Several workers have done similar work with amphibians.[87]

Von Hippel [88] produced cataracts in rabbit embryos by roentgen irradiation of the pregnant mother. Pagenstecher [89] obtained rosette formation in the retina by a similar procedure. Comparable effects have been observed in human embryos.[90] Experimentation with pregnant rats has yielded the following observations [91]: Hydrocephalus resulted most frequently from irradiation on the ninth day; ocular abnormalities, from that on the tenth day, and malformations on the jaws, from that on the eleventh day. In mice, irradiation on the seventh day resulted in resorption of embryos; that on the eighth day in meningocele; that on the ninth to fourteenth day, in kinked or short tail; that on the twelfth to fourteenth day, in hydrocephalus; that on the fourteenth to seventeenth day, in sterility, and that on the eighteenth and nineteenth day, in cataract.[92] This differential action may well be due to an effect of radiation proportionate to the growth rate at a given time and place,[93] although many other factors are probably involved as well. That roentgen rays have their predominant effect on cells in mitosis is well established.[14] A combination of irradiation and administration of ether [94] or increase of temperature [16b] has been reported to increase the effect in experimental animals.

Much has been reported on human embryos that received therapeutic doses of roentgen rays. In contrast to irradiation of the germ cells, which has not been proved to affect the offspring, irradiation of the embryo is responsible for a large number of cases of maldevelopment, which have been thoroughly investigated, notably microcephaly with

86. Solberg, A. N.: J. Exper. Zool. **78**:441, 1938.

87. Bardeen,[16b] Hertwig,[16c] Baldwin, W. M.: Anat. Rec. **17**:135, 1919; Am. J. Physiol. **52**:296, 1920; Anat. Rec. **22**:305, 1921. Curtis, W. C.; Cameron, J. A., and Mills, K. O.: Science **83**:354, 1936.

88. von Hippel, E.: Verhandl. d. deutsch. path. Gesellsch. **9**:174, 1905.

89. Pagenstecher, H. E.: Ber. ü. d. Versamml d. ophth. Gesellsch., 1916, p. 447.

90. (*a*) Lindenfeld, B.: Klin. Monatsbl. f. Augenh. **51**:443, 1913. (*b*) Goldstein, I., and Wexler, D.: Arch. Ophth. **5**:591, 1931; (*c*) **7**:434, 1932.

91. Job, T. T.; Leibold, G. L., Jr., and Fitzmaurice, H. A.: Am. J. Anat. **56**:97, 1935.

92. Kaven, A.: Ztschr. f. menschl. Vererb.- u. Konstitutionslehre **22**:238, 1938.

93. Woskressensky, N. M.: Arch. f. Entwcklngsmechn. d. Organ. **113**:447, 1928.

94. Haecker, V., and Lebedinsky, N.: München. med. Wchnschr. **61**:7, 1914.

mental deficiency and less frequently hydrocephalus, microphthalmia, malformations of the extremities and other parts.[95] Retinal rosette formations comparable to those experimentally produced in the rabbit [89] have been studied in detail in embryos irradiated in order to terminate pregnancy.[90] It has been found that well over 50 per cent of irradiated embryos (not counting those irradiated for intentional termination of pregnancy) suffer severe damage.[95b, c] In view of this fact it has been suggested that pregnancy be interrupted if through unfortunate circumstances the embryo has received therapeutic doses of roentgen rays.[95a] Diagnostic curettage of the uterus before irradiation of the organ during the generative period has also been advocated.[95c] While in most of the reported cases the damage of the embryo was due to early irradiation, ill effects have been observed to follow treatment given during the latter half of intrauterine life.[96] According to Bagg [97] and Goldstein and Murphy,[98] the effect of radium resembles that of roentgen rays. It need not be elaborated in detail that these malformations are not hereditary, in contrast to those caused by mutation after irradiation of germ cells (see page 404).

Chemical Influences.—Countless investigations have dealt with chemical alterations of the embryo. Only a few will be mentioned for their biologic or medical significance. Much of the early work was done on the fish Fundulus heteroclitus, and striking modifications of development were obtained. Werber [99] produced double monsters by means of acetone. In addition, he [100] obtained, by administration of acetone or butyric acid, cyclopia and various malformations of the eyes, ears, olfactory pits, mouth, central nervous system, heart and vessels, fins, tail and body form. There are also most interesting cases in which isolated eyes or lenses were found on the blastoderm far from the embryo itself.[101] This led Werber [101b] to develop the concept of blastolysis, which means an action of chemicals resulting in destruction or in

95. (*a*) Murphy, D. P.; Shirlock, M. E., and Doll, E. A.: Am. J. Roentgenol. **48**:356, 1942. (*b*) Zappert, J.: Wien. klin. Wchnschr. **38**:669, 1925; Arch. f. Kinderh. **80**:34, 1926. (*c*) Murphy, D. P.: Am. J. Obst. & Gynec. **18**:179, 1929; (*d*) footnote 21. (e) Feldweg, P.: Strahlentherapie **26**:799, 1927. (*f*) Goldstein, L., and Murphy, D. P.: Surg., Gynec. & Obst. **50**:79, 1930. (*g*) Flaskamp, W., in Meyer, H.: Sonderbünde zur Strahlentherapie, Berlin, Urban & Schwarzenberg, 1930, vol. 12, p. 1. (*h*) Sternberg, H.: Chir. d. org. di movimento **24**:231, 1939. (*i*) Maxfield, F. N.: Am. J. Ment. Deficiency **45**:358, 1941. (*j*) Glass, S. J.: J. Clin. Endocrinol. **4**:47, 1944.

96. Goldstein, L., and Murphy, D. P.: Am. J. Roentgenol. **22**:322, 1929.

97. Bagg, H. J.: Am. J. Anat. **30**:133, 1922.

98. Goldstein, L., and Murphy, D. P.: Am. J. Obst. & Gynec. **18**:189, 1929.

99. Werber, E. I.: J. Exper. Zool. **24**:409, 1917.

100. Werber, E. I.: Anat. Rec. **9**:529, 1915; footnote 6 *e*.

101. Werber, E. I.: (*a*) J. Exper. Zool. **21**:347, 1916; (*b*) Anat. Rec. **10**:258, 1916.

splitting and dispersion of the germ. As this work was based on experimental use of acetone and butyric acid, which may be present in the human circulation under abnormal conditions, it was suggested [102] that human malformations may have the same cause. While this deduction cannot be subscribed to, for many obvious reasons, and the mechanism of Werber's blastolysis is not understood, the interesting facts remain and have been powerful stimuli for further work. Stockard used inorganic salts in experiments on the same species and found that magnesium salts produced a remarkable incidence of cyclopia. The most constant effect on various embryonic structures was obtained with lithium salts. These produced a retardation of development, malformations of the eyes, colorless (sic) blood and a slow heart rate.[103] McClendon [6d] examined the power of many inorganic and organic compounds to produce cyclopia, and along with the chemical properties of these agents he considered also their surface tension.

In regard to amphibians the work done with lithium salts overshadows all other experiments with chemicals in extent and importance. Lehmann [104] found the notochord absent and the myotomes (somites) fused across the midline in locations depending on the times at which the subjects were exposed to the salts. Similar results were obtained by Cohen,[105] and the original articles should be consulted for the somewhat different explanations given by the two workers. In the head region, not only faulty differentiation or arrangement of the primordia occurs, but severe defects up to cyclopia or complete anophthalmia with associated lesions of the brain. All these malformations are not specific for the action of lithium; they occur, though less regularly, after the use of other chemicals [106] and after an increase of temperature.[107] The action of trichlorbutyl-alcohol on lens formation in amphibians has also been investigated in great detail and with particular attention to the effect of various concentrations.[108]

An interesting and severe malformation in amphibians, which occasionally occurs spontaneously, has been produced with increased frequency by raising embryos in 0.35 per cent sodium chloride solution.[109] It is exogastrulation, an abnormality of the process of gastrulation in which the mesoderm, instead of moving into the interior at the blastopore, moves outward to form a separate mass. In extreme

102. Werber, E. I.: Anat. Rec. **9**:133, 1915; Bull. Johns Hopkins Hosp. **26**:226, 1915; footnote 6 *e*.

103. Stockard, C. R.: J. Exper. Zool. **3**:99, 1906; footnote 6 *a*.

104. Lehmann, F. E.: Arch. f. Entwcklngsmechn. d. Organ. **136**:112, 1937; **136**:106, 1938.

105. Cohen, A.: J. Exper. Zool. **79**:461, 1938.

106. Lehmann.[104] Cohen.[105]

107. Hoadley, L.: Growth **2**:25, 1938.

108. Lehmann, F. E.: Arch. f. Entwcklngsmechn. d. Organ. **131**:333, 1934.

109. Holtfreter, J.: Arch. f. Entwcklngsmechn. d. Organ. **129**:669, 1933.

cases it may constrict itself off from the ectoderm, and the embryo is then divided into two separate pieces. Just what the action of the salt is in provoking this process is not understood. Exogastrulation has also appeared occasionally after other experimental procedures.

In frog embryos 2,4-dinitrophenol produces a general retardation of development and any of the following malformations: persistent yolk plug, absence of external gills, papillary outgrowths of the epidermis and abnormalities of the eyes and the neural tube.[110]

There are many reports on the action of chemicals on the chick embryo. Various substances acting chemically or as foreign bodies were administered by Bauer [111] and Canat and Opie.[112] The former reports hypoplasia of the mesenchyme and inhibition of the outgrowth of peripheral nerves, supposedly consecutive to the disturbance of the mesenchyme. Ectodermal and entodermal structures are normal or retarded, or show foci of necrosis. Canat and Opie examined the local inflammatory reaction to the injection of india ink or turpentine. In embryos of 3 to 5 days the most prominent reaction is cell proliferation. Phagocytosis also occurs early. Granulocytes begin to appear at the end of the first week. Shortly before birth, inflammation assumes the well known postnatal forms. That scarlet red induces epithelial proliferations and irregularities of the neural tube has been claimed,[113] but not confirmed in a later investigation.[114] It is not known whether or not this discrepancy was due to differences in the chemical nature of the dye or to its impurities. Alcohol in suitable concentrations produces tachycardia in 48 hour embryos, but no malformations beyond disturbances of the curvature of the body.[115] Colchicine produces not only the well known abnormalities of mitosis ("colchicine figures") but also malformations of the neural tube [116] or, according to other authors,[117] strophosomus (an extreme dorsal flexion of the spine). When colchicine is applied to certain circumscribed parts of the embryo, dwarfed limbs may be obtained, as well as reduced numbers of digits.[118] Landauer [119] observed in chick embryos, after injecting Ringer solution into the eggs, an increased prenatal and a decreased postnatal mortality. This is

110: Dawson, A. B.: J. Exper. Zool. **78**:101, 1938.

111. Bauer, K.: Virchows Arch. f. path. Anat. **294**:477, 1935.

112. Canat, E. H., and Opie, E. L.: Am. J. Path. **19**:371, 1943.

113. Waelsch, L.: Arch. f. Entwcklngsmechn. d. Organ. **38**:509, 1914. I had an opportunity to see serial sections of Waelsch's specimens and to confirm the presence of the malformations described in this article.

114. Burnier, J., and Sauser-Hall, P.: Compt. rend. Soc. de biol. **116**:927, 1934.

115. Petry, E., and Ferrier, A.: Compt. rend. Soc. de biol. **116**:928, 1934.

116. Paff, G. H.: Am. J. Anat. **64**:331, 1939.

117. Lallemand, S.: Compt. rend. Acad. d. sc. **207**:1446, 1938. Ancel, P., and Lallemand, S.: ibid. **210**:710, 1940. Gabriel, M. L.: J. Exper. Zool. **101**:339, 1946.

118. Gabriel, M. L.: J. Exper. Zool. **101**:339, 1946.

119. Landauer, W.: Poultry Sc. **8**:301, 1929.

apparently an instance in which mostly the weaker ones, which would succumb to other influences after birth, are killed as embryos. As was mentioned in a foregoing paragraph, a similar explanation has been given for the action of alcohol on the germ cells.[81]. Landauer [119] also found that lithium salts and, to a less extent, magnesium salts cause a great increase of mortality shortly before hatching, but no malformations were seen. In another series of experiments, rumplessness was produced by insulin and several other organic compounds.[120] Catizone and Gray [121] have reported three types of distortion of the head following administration of lead compounds. The published views of whole embryos suggest that the malformations are all platyneuria, which occurs frequently in the laboratory without intentional interference. Other workers [122] found general retardation of growth and relatively greater retardation of somite formation. The development of head and eyes is inhibited. In both investigations the embryos were not sectioned. Gray and Worthing [123] injected tetanus toxin and observed a profound influence on the central nervous system and the head of the early embryo. These malformations, too, are of those types which frequently occur without apparent cause.

Of great economic importance is a series of investigations originally designed to discover the cause of so-called alkali disease of farm animals in the Middle West.[124] It was found that the disease is a poisoning due to consumption of selenium-containing plants. In connection with the present subject, only the findings in chick embryos are of interest. Mature chickens are not much affected by selenium in their diet, but embryos of selenium-poisoned hens show severe disturbances.[125] A high percentage of them fail to hatch and exhibit, among others, malformations of the brain, the eyes (including cyclopia), the beak and the extremities or an "edemic" (sic) condition. Similar malformations have been produced by injecting selenium compounds into eggs.[126] In the course of the same investigation arsenic, fluorine and lead compounds were also tested and failed to cause comparable disturbances. These substances caused a high embryonic death rate, also ectopia of the viscera.[126] Landauer [49] found that selenium-induced malformations share with certain others a predilection for one side of the body (eyes and wings of the left side; legs of the right side).

120. Landauer, W.: J. Exper. Zool. **98**:65, 1945. Landauer, W., and Lang, E. H.: ibid. **101**:41, 1946. Landauer, W., and Bliss, C. I.: ibid. **102**:1, 1946.

121. Catizone, O., and Gray, P.: J. Exper. Zool. **87**:71, 1941.

122. Hammett, F. S., and Wallace, V. L.: J. Exper. Med. **48**:659, 1928.

123. Gray, P., and Worthing, H.: J. Exper. Zool. **86**:423, 1941.

124. Moxon, A. L.: Bulletin 311, South Dakota Agricultural Experiment Station, 1937, p. 1.

125. Franke, K. W., and Tully, W. C.: Poultry Sc. **14**:273, 1935.

126. Franke, K. W.; Moxon, A. L.; Poley, W. E., and Tully, W. C.: Anat. Rec. **65**:15, 1936.

Few investigations have been undertaken to examine in mammals the influence on the embryo of substances injected into pregnant females. In recent years drugs have been tested in order to determine whether, if used during pregnancy, they might endanger the embryo. Sulfanilamide was tested in rats.[127] The concentration was equal in the maternal and the fetal blood and higher than that used in therapy. Prolonged administration increased the mortality before and after birth. The birth weight was diminished and postnatal growth retarded. Penicillin, on the other hand, had no detrimental effect on the embryo under the conditions of an independent investigation.[128] Thiourea was similarly examined.[129] The thyroid gland of the embryo showed the histologic changes characteristic of thiourea treatment. No malformations were recorded. Strontium compounds administered to pregnant rabbits replace calcium in fetal bones and produce a condition of "pseudorickets".[130] In rat embryos whose mothers receive a diet containing 25 per cent galactose cataracts develop. The changes resemble postnatal galactose cataract.[131] Alloxan has no direct effect on the rat embryo.[132] Hansmann and Perry [133] in a series of unselected cases in which there was no exposure to industrial hazards found lead in 62.5 per cent of human fetuses examined. In 25 per cent the amounts were considered dangerous. However, it is stated that the fetus is protected by the growing skeleton which binds the lead. The authors emphasize the possibility of abortion due to lead. No malformations were observed. Only one report has been found concerning the possibility that the fetus might be damaged by arsenicals used in antisyphilitic treatment during pregnancy.[134]

In the preceding pages the effects of foreign substances on embryonic development are reviewed. It is to be expected that striking effects would be produced in the embryo by those organic substances which even in the more stabile mature organism control structural changes, namely, hormones. It is unfortunate that Gley's [135] subdivision of these

127. Speert, H.: Bull. Johns Hopkins Hosp. **66**:139, 1940.

128. Greene, H. J., and Hobby, G. L.: Proc. Soc. Exper. Biol. & Med. **57**:282, 1944.

129. Goldsmith, E. D.; Gordon, A. S., and Charipper, H. A.: Am. J. Obst. & Gynec. **49**:197, 1945.

130. Lehnerdt, F.: Beitr. z. path. Anat. u. z. allg. Path. **46**:468, 1909.

131. Bannon, S. I.; Higginbottom, R. M.; McConnell, J. M., and Kaen, H. W.: Arch. Ophth. **33**:224, 1945.

132. Friedgood, C. E., and Miller, A. A.: Proc. Soc. Exper. Biol. & Med. **59**:61, 1945.

133. Hansmann, G. H., and Perry, M. C.: Arch. Path. **30**:226, 1940.

134. Arnold, W.: Virchows Arch. f. path. Anat. **311**:1, 1944.

135. Gley, E.: The Internal Secretions: Their Physiology and Application to Pathology, New York, Paul B. Hoeber, 1917.

active substances into hormones proper and harmozones, proposed in the early days of endocrinology, has not been generally accepted. According to Gley's definition, substances of the former group control functional activity, whereas harmozones control morphogenetic processes. Disturbances in the balance of harmozones should affect the embryo profoundly. The relationship of these agents to embryonic organizers has been discussed by Needham.[4]

The older literature on the endocrine glands of the fetus and their interrelation with one another, with other parts of the embryo and with agents in its environment has been reviewed by Thomas.[136] One important concept dating from that early period is that of synkainogenesis,[137] a term which designates all endocrine interactions between mother and embryo. Well known examples of this are the stimulation of the mammary glands of the newborn by maternal lactogenic hormone and the hyperplasia of the interstitial cells of the testes of the embryo, which is well marked in man [138] and reaches tremendous proportions, also those of the ovaries, in horse embryos.[139] Endocrine abnormalities of the mother may influence the embryo. Peculiarities of the children of diabetic mothers have recently been studied in great detail. They include increased birth weight, enlargement of the heart, extramedullary erythropoiesis, hyperplasia of the pancreatic islands and changes in other endocrine organs.[140] It was thought that the metabolic disturbance and the insulin deficiency of the mother were directly responsible for these changes. However, it has now been found that infants of mothers in whom diabetes develops only some time after termination of pregnancy have similar changes.[141] This has not been explained as yet. The fetal and neonatal mortality is increased during a five year period preceding the onset of maternal diabetes.[142]

Hereditary dwarfism of mice, apparent at birth, is probably mediated by an abnormality of the pituitary gland.[143] Mongoloid deficiency has been tentatively correlated with maternal and fetal pituitary dysfunc-

136. Thomas, E.: Innere Sekretion in der ersten Lebenszeit (vor und nach der Geburt), Jena, Gustav Fischer, 1926.

137. Kohn, A.: Arch. f. Entwcklngsmechn. d. Organ. **39**:112, 1914.

138. Mita, G.: Beitr. z. path. Anat. u. z. allg. Path. **58**:554, 1914. Neumann, H. O.: Ztschr. f. Geburtsh. u. Gynäk. **90**:100, 1930. Diaca, C.: Virchows Arch. f. path. Anat. **304**:171, 1939.

139. Kohn, A.: Ztschr. f. Anat. u. Entwcklngsgesch. **79**:366, 1926. Petten, J. L.: ibid. **99**:338, 1933.

140. (*a*) Potter, E. L.; Seckel, H. P. G., and Stryker, W. A.: Arch. Path. **31**:467, 1941. (*b*) Miller, H. C.; Johnson, R. D., and Durlacher, S. H.: J. Pediat. **24**:603, 1944.

141. Miller, H. C.: Am. J. M. Sc. **209**:447, 1945.

142. Miller, H. C.: J. Pediat. **29**:455, 1946.

143. Greene, H. S. N.: J. Exper. Med. **71**:839, 1940.

tion.[144] On the other hand, human subjects with pituitary dwarfism usually produce normal offspring.[145] Fetal thyroid or iodine deficiency produces congenital cretinism.[146] The mental development of persons with congenital hypothyroidism, particularly that of endemic cretins, often fails to respond adequately to administration of thyroid. It has therefore been suggested that damage of the brain may not be merely a consequence of thyroid deficiency but an associated lesion.[147]

A possible correlation of abnormalities of endocrine organs of the embryo is the repeatedly investigated severe atrophy of the adrenal cortex of the anencephalic monster.[148] It has not been definitely established whether or not this condition is caused by an abnormality of the hypophysis in the anencephalic monster, as was claimed by Kohn.[148b] I have seen a similar atrophy of the adrenal cortex in a stillborn infant with hydrocephalus and abnormalities at the base of the brain. No pituitary gland could be found (case unpublished).

By far the largest amount of work in embryonic endocrinology has been done with estrogenic and androgenic substances. It is of great interest because many of the experiments have resulted in abnormalities related to intersexuality. Work done up to 1939 has been reviewed by a group of outstanding experts.[64] Since then, experimental work has been done with the opossum,[149] the mouse,[150] the rat[151] and the monkey.[152] In all classes of vertebrates the genital organs and other sexually different characteristics may be transformed into forms more or less resembling those of the opposite sex by proper application of estrogen or androgen to embryos. A strong tendency of the genetic sex to assert itself and condition the effect of estrogen or androgen was noted in some of these experiments but until recently hormones were generally

144. (*a*) Benda, C. E.; Dayton, N. A., and Prouty, R. A.: Am. J. Psychiat. **99**:822, 1943. (*b*) Beidleman, B.: Am. J. Ment. Deficiency **50**:35, 1945.

145. Speck, G.: Am. J. Obst. & Gynec. **51**:217, 1946.

146. Benda, C. E.: Mongolism and Cretinism, New York, Grune & Stratton, Inc., 1946.

147. Bruch, H., and McCune, D. J.: Am. J. Dis. Child. **67**:205, 1944.

148. (*a*) Meyer, R.: Virchows Arch. f. path. Anat. **210**:158, 1912. Landau, M.: Verhandl. d. deutsch. path. Gesellsch. **16**:301, 1913. (*b*) Kohn, A.: Arch. f. Entwcklngsmechn. d. Organ. **102**:113, 1924. Angevine, D. M.: Arch. Path. **26**:507, 1938.

149. (*a*) Moore, C. R.: Physiol. Zoöl. **14**:1, 1941. (*b*) Burns, R. K., Jr.: Biol. Symposia **9**:125, 1942.

150. Raynaud, E.: Compt. rend. Acad. d. sc. **205**:1453, 1937; Compt. rend. Soc. de biol. **127**:503, 1938. Turner, C. D.: J. Morphol. **65**:353, 1939.

151. (*a*) Hamilton, J. B., and Wolfe, J. M.: Anat. Rec. **70**:433, 1938. (*b*) Greene, R. R.; Burrill, M. W., and Ivy, A. C.: Am. J. Anat. **65**:415, 1939; **67**:305, 1940. (*c*) Greene, R. R.: Biol. Symposia **9**:105, 1942.

152. van Wagenen, C., and Hamilton, J. B., in Essays in Biology in Honor of Herbert M. Evans, Berkeley, University of California Press, 1943, p. 581.

believed to play a dominating part in the morphogenesis of the genital organs (except the gonads themselves) under normal as well as under abnormal conditions. From experiments carried out during the last few years it has been concluded by some leading authorities that the gonads of the embryo do not produce hormones until after the genital tract is well established in male or female form. Even the gonadotropic hormone of the pituitary gland fails to stimulate the gonads in these stages to secrete hormones.[153] This is in accord with pathologic findings in human cases of congenital absence or severest hypoplasia of the gonads [154] in which the genital tract is definitely differentiated in the direction of one sex. In later periods sexual differentiation will, of course, suffer in gonadless individuals. The power of the genetic sex to assert itself, even in postnatal periods, is well illustrated in experiments in which male chick embryos were completely feminized by administration of estrogen. They had histologically normal ovaries, and yet were reconverted to the male sex after birth if estrogen treatment was not continued.[155] Thus the leading role of the genetic sex in determining not only the sexual form of the gonads but also that of the entire organism has come to be increasingly appreciated.

It is of great medical importance to realize the tenacity of the genetic sex. It explains the fundamental difference between genetic and hormonal intersexuality. In the former the sexual development is abnormal by the standards of the usual male or female organization but normal for the individual itself in that it conforms with that individual's genetic pattern. Hormonal intersexes, on the other hand, have a sex which is genetically normal by the usual standards but which is secondarily changed by hormones. These individuals will by themselves tend to revert to their genetic sex if the hormonal imbalance is eliminated. In addition, there is probably a condition of genetically determined hormonal intersexuality which may, if not properly analyzed, be confused with genetic intersexuality. It is produced by the action of genetically determined hormonal imbalance in individuals with a normal genetic sex. This occurs in genetically caused or conditioned growths with heterosexual hormonal activity. Familial intersexuality associated with hyperplasia or tumor of the adrenal cortex [156] probably belongs to this group.

153. Moore, C. R.: Am. Naturalist **78**: 97, 1944; J. Clin. Endocrinol. **4**:135, 1944.

154. Kermauner, F., in Halban, J., and Seitz, L.: Biologie und Pathologie des Weibes, Berlin, Urban & Schwarzenberg, 1924, vol. 3, p. 281. Rössle, R., and Wallart, J.: Beitr. z. path. Anat. u. z. allg. Path. **84**:401, 1930. Pich, G.: ibid. **98**:218, 1937. Erskine, C. A., and Rannie, I.: Arch. Path. **42**:381, 1946.

155. Wolff, E.: Arch d'anat., d'histol. et d'embryol. **23**:1, 1936. Dantchakoff, V.: Compt. rend. Acad. d. sc. **202**:1112, 1936.

156. Werthemann, A.: Schweiz. med. Wchnschr. **71**:1335, 1941.

While the domain of the sex hormones proper may not be as great in normal embryonic development as was formerly believed, it is quite possible that chemical correlations, perhaps more of the organizer type, are active in the early development of the genital organs. Here must be mentioned Witschi's [157] hypothetic substances which govern female development of the germ cells in the cortex, and male development in the medulla, of the gonad. Of undetermined normal significance is a hormone-like substance which produces a highly perplexing type of intersexuality in certain mammals: The freemartins among cattle are individuals with abnormal sex development, which are always twins of normal males. It was discovered simultaneously and independently by Keller and Tandler [158] and Lillie [159] that the abnormal twin is a genetic female modified by a substance transmitted from the male twin through anastomoses in their chorionic circulations. A considerable number of detailed investigations have since substantiated this theory and elucidated the development of the freemartin. Similar intersexual twins also occur among pigs and goats but not among heterosexual twins with vascular anastomoses in the cat, the peludo and the marmoset (see Witschi's review [160]). Moore [158] points out that conditions strictly comparable with those observed in the freemartin have not as yet been produced in experiments, and he concludes that the problem of the freemartin is just as mysterious now as ever. Marsman [161] compared freemartins with genetic intersexes and geldings as to morphologic aspects and excretion of hormones. His conclusion is that the freemartin is an individual deprived early of its sources of sex hormones and more thoroughly than the gelding.

It remains to mention the effect of certain tumors of the cortex of the adrenal gland on the genital tract. In a small proportion of cases of corticoadrenal tumors for which no specific histologic characteristics have yet been found, as well as in some instances of hyperplasia of the adrenal cortex, the female subjects may be masculinized at any period of prenatal or postnatal life (interrenalism [162]). This effect disappears promptly on removal of the excessive adrenal tissue unless sex transformation in early embryonic life has produced irreversible changes.[163] A much smaller number of cases in which men were

157. Witschi, E.: Biol. Rev. **9**:460, 1934.

158. Keller, K., and Tandler, J.: Wien. tierärztl. Wchnschr. **3**:513, 1916.

159. Lillie, F. R.: Science **43**:611, 1916.

160. Witschi, E., in Allen, Danforth and Doisy,[64] p. 145.

161. Marsman, W. S.: Acta neerland. morphol. **1**:115, 1937.

162. Mathias, E.: Zentralbl. f. Gynäk. **50**:2489, 1926. Berner, O., in Hirsch, M.: Handbuch der inneren Sekretion, Leipzig, Curt Kabitzsch, 1927, vol. 25, p. 1143.

163. McKenna, C. M.; Kiefer, J. H., and Bronstein, I. P.: Tr. Am. A. Genito-Urin. Surgeons **35**:41, 1943.

feminized by similar growths have been reported.[164]. Familial occurrence has already been mentioned.[156] Various theories have been proposed concerning the androgenic activity (neglecting the few cases in which feminization occurred) of one or another of the normal constituents of the adrenal cortex and he relationship of the just mentioned tumors to these. None of the theories is supported by convincing evidence, and they will therefore not be reviewed. Somewhat less problematic is the influence on sex of various tumors of organs which normally produce sex hormones, as the testis, the ovary and the placenta. This subject is adequately treated in many texts of pathology or of gynecology. A case of masculinization of a female fetus by an ovarian tumor of the mother, probably an arrhenoblastoma, is on record.[165]

Deficiencies.—If any of essential substances is lacking during embryonic life, this may produce severe developmental disturbances. Some of these disturbances have been investigated in chick embryos, partly because their occurrence in commercial hatching is of considerable economic importance. Micromelia was observed in chick embryos, caused by a deficient diet of the hen, by Byerly and co-workers,[166] and its morphologic aspects were studied by Landauer.[167] Lyons and Insko[168] and Caskey and Norris[169] have described the same manifestations in manganese deficiency and they, and later Landauer,[170] have assumed that the first-mentioned deficiency was also one of manganese. Gallup and Norris[171] found that manganese deficiency produces increased mortality of chick embryos. The embryos are fully developed but unable to hatch. The authors call this congenital debility in analogy to findings in the rat (see page 428). The influence of vitamin deficiencies has been examined in chick embryos. Low vitamin A content of the egg results in poor hatchability.[172] Riboflavin deficiency causes degeneration of the mesonephros, edema, anemia and abnormal down.[173] Another report mentions lethal changes in

164. Schiller, W.: Internat. Clin. **3**:87, 1940.

165. Brentnall, C. P.: J. Obst. & Gynaec. Brit. Emp. **52**:235, 1945.

166. Byerly, T. C.; Titus, H. W.; Ellis, N. R., and Landauer, W.: Proc. Soc. Exper. Biol. & Med. **32**:1542, 1935.

167. Landauer, W.: Anat. Rec. **64**: 267, 1936.

168. Lyons, M., and Insko, W. M., Jr.: Bulletin 371, Kentucky Agricultural Experiment Station, 1937, p. 61.

169. Caskey, C. D., and Norris, L. C.: Proc. Soc. Exper. Biol. & Med. **44**:332, 1940.

170. Landauer, W.: Sigma Xi Quart. **28**:171, 1940.

171. Gallup, W. D., and Norris, L. C.: Poultry Sc. **18**:83, 1939.

172. Bearse, G. E., and Miller, M. W.: Poultry Sc. **16**:39, 1937.

173. Lepovsky, S.; Taylor, L. W.; Jukes, T. H., and Almquist, H. L.: Hilgardia **11**:559, 1938.

the extraembryonic vessels ("lethal ring") and chondrodystrophy and other defects in those embryos which survive.[174] A third group[175] claims that the first changes to appear are those in the nervous system, including degeneration of myelin sheaths. These can be prevented by injecting riboflavin into the eggs. Vitamin D deficiency seems to occur in chick embryos as indicated by the high mortality in eggs of hens kept in closed rooms, which can be reduced by giving the hens cod liver oil.[176] Vitamin E deficiency causes retardation of development, and death through degeneration of vessels, rupture of the atrium of the heart or of large vessels, and impairment of growth of the allantois, which is essential for respiration.[177] However, the pertinence of these observations has been questioned,[178] because it is not certain that they are the effect of vitamin E deficiency alone. Lack of biotin is followed by increased embryonic mortality at certain stages, chondrodystrophy and syndactyly.[179]

Considerable experimental work has been reported on the effect of vitamin deficiencies on mammalian embryos. Many references have been gathered by Mason.[180] The same author has reported in detail on the results of vitamin A deficiency in pregnant rats[181]: Early death of many embryos, followed by resorption, results from inflammatory changes of the placenta. The surviving embryos are retarded in growth, gestation is often unduly prolonged, and many newborn young die soon. In the pig severe defects of the hindlegs follow maternal deficiency of a fat-soluble factor,[182] which Needham[4] states is vitamin A. In another investigation definite lack of this vitamin caused microphthalmia in every young one throughout several experiments, but no malformations of the legs.[183] In the rat, ocular malformations of a different kind are observed in association with maternal vitamin A deficiency.[184] A series of reports describes the development of cleft palate and malformations of the extremities of the embryos of rats

174. Romanoff, A. L.: Anat. Rec. (supp.) **78**:78, 1940.

175. Engel, R. W.; Phillips, P. H., and Halpin, J. G.: Poultry Sc. **19**:135, 1940.

176. Insko, W. M., and Lyons, M.: Bulletin 363, Kentucky Agricultural Experiment Station, 1936, p. 64.

177. Adamstone, F. B.: J. Morphol. **52**:47, 1931.

178. Mason, K. E.: Yale J. Biol. & Med. **14**:605, 1942.

179. Cravens, W. H.; McGibbon, W. H., and Sebesta, E. E.: Anat. Rec. **90**:55, 1944.

180. Mason, K. E., in Allen, Danforth and Doisy,[64] p. 1149.

181. Mason, K. E.: Am. J. Anat. **57**:303, 1935.

182. daZilva, S. S.; Golding, J.; Drummond, J. C., and Coward, K. H.: Biochem. J. **15**:427, 1921.

183. Hale, F.: Texas State J. Med. **33**:228, 1937.

184. Warkany, J., and Schraffenberger, E.: Proc. Soc. Exper. Biol. & Med. **57**:49, 1944; Arch. Ophth. **35**:150, 1946.

maintained on a certain deficient diet.[185] These disturbances can be prevented with dried pig liver. The same malformations result from riboflavin deficiency.[186] Similar, but not identical, defects were produced by feeding the mother a rachitogenic diet.[187] Avitaminosis C (deficiency of ascorbic acid) has been produced in guinea pig embryos.[188] Research on the effect of vitamin E deficiency started with the work of Evans, Burr and Althausen [189] and Urner.[190] Early abnormal development of the mesenchyme and general retardation were observed, as well as reduction of the entodermal villi of the placenta and impairment of the blood islands. The result was death of the fetus. Mason [191] described in hypovitaminotic embryos which survived to the sixteenth day a hemorrhagic state, with much blood accumulated in the vessels of the skin, and cerebral hemorrhages. He attributes the lack of cells in the blood-forming tissues to this escape of blood to the periphery.

Manganese deficiency produces in rats a state of "congenital debility" which renders them unable to live through the period of birth, even though they appear normal in utero shortly before term.[192]

Vitamin deficiencies of human embryos and newborn infants have repeatedly been reported.[193] In most cases neither the type of deficiency nor that of the structural changes has been clearly defined. The conditions found were called fetal rickets or osteomalacia, but no typical entities have as yet been demonstrated. The possible significance of vitamin deficiencies is indicated by the suggestion of Balfour and Talpade [194] that the high infant mortality of India may be caused by a deficiency, possibly of the vitamin B complex. Lack of iron in tuber-

185. Warkany, J., and Nelson, R. C.: Anat. Rec. **79**:83, 1941; Arch. Path. **34**:375, 1942. Warkany, J.; Nelson, R. C., and Schraffenberger, E.: Am. J. Dis. Child. **64**:860, 1942.

186. Warkany, J., and Schraffenberger, E.: Proc. Soc. Exper. Biol. & Med. **54**:92, 1943.

187. Warkany, J.: Am. J. Dis. Child. **66**:511, 1943.

188. (*a*) Ingier, A.: J. Exper. Med. **21**:525, 1915. (*b*) Reyher, P.; Walkhoff, E., and Walkhoff, O.: München. med. Wchnschr. **75**:2087, 1928.

189. Evans, H. M.; Burr, G. O., and Althausen, T. L.: The Antisterility Vitamine Fat Soluble E, Memoirs of the University of California, Berkeley, University of California Press, 1927, vol. 8, p. 1.

190. Urner, J. A.: Anat. Rec. **50**:175, 1931.

191. Mason, K. E., in Essays in Biology in Honor of Herbert M. Evans, Berkeley, University of California Press, 1943, p. 401.

192. Daniels, A. L., and Everson, J. G.: J. Nutrition **9**:191, 1935.

193. (*a*) Reyher, Walkhoff and Walkhoff,[188b] (*b*) Maxwell, J. P.; Hu, C. H., and Turnbull, H. M.: J. Path. & Bact. **35**:419, 1932. (*c*) Akamatu, K.: Okayama-Igakkai-Zasshi **52**:979, 1940.

194. Balfour, M. I.: Indian M. Gaz. **65**:630, 1930. Balfour, M. I., and Talpade, S. K.: ibid. **67**:601, 1932.

culous mothers and their fetuses has been suggested as the cause of anemia and decreased resistance of the newborn.[195] Iodine deficiency produces in mammals congenital goiter and hairlessness[196] and in man goiter and deaf-mutism.[197] Stott[198] attributes similar findings to an excess of calcium in the water rather than to iodine deficiency, or to a disproportion of calcium and iodine. Closely related conditions were mentioned in a foregoing section under thyroid deficiency.

Detrimental effects of an unspecified nutritional deficiency of the mother on the fetus are suggested by Burke, Beal, Kirkwood and Stuart,[199] who state that in 216 cases studied, "every stillborn infant, every infant who died within a few days of birth, with the exception of one, the majority of infants with marked congenital defects, all premature and all 'functionally immature' infants were born to mothers whose diet during pregnancy was very inadequate." In view of the large number of other known causes of the conditions in question, one may not agree with this statement to its full extent; yet, it is perhaps not quite true that the fetus always obtains the best possible "diet" even at the expense of the mother, as has often been stated.

Changes in Concentration of Oxygen and Carbon Dioxide.—If the concentrations of oxygen and carbon dioxide in the environment of incubating chicken eggs are decreased or increased, respectively, beyond certain levels this interferes with normal development of the embryo. According to Romanoff,[200] concentrations of carbon dioxide up to 0.4 per cent are compatible with normal development and may even stimulate growth. According to the same investigator, concentrations of carbon dioxide higher than 1 per cent with a corresponding reduction of oxygen produce slow growth, various malformations and early death. If the exposure to air of abnormal composition is temporary, concentrations up to 22 per cent carbon dioxide with 16 per cent oxygen are compatible with continued growth. Another author[201] obtained a high percentage of embryos with platyneuria (a peculiar form of nonclosure of the neural tube) by preventing the exchange of gas through the egg shell with a coat of wax. In other experiments embryos exposed to an atmosphere containing 22 to 37 per cent

195. Albers, H.: Arch. f. Gynäk. **172**:173, 1941.

196. Smith, G. E.: J. Biol. Chem. **29**:215, 1917. Welch, K.: Bulletin 119, University of Montana Agricultural Experiment Station, 1917, p. 81. Hart, E. B., and Steenbock, H.: J. Biol. Chem. **33**:313, 1918.

197. Brain, W. R.: Quart. J. Med. **20**:303, 1927. Murray, M. M., and Wilson, D. C.: Nature, London **155**:79, 1945.

198. Stott, H.: Indian J. M. Research **20**:147, 1932.

199. Burke, B. S.; Beal, V. A.; Kirkwood, S. B., and Stuart, H. C.: Am. J. Obst. & Gynec. **46**:38, 1943.

200. Romanoff, A. L.: J. Morphol. **50**:517, 1930.

201. Gallera, J.: Folia morphol. **6**:203, 1936.

carbon dioxide presented platyneuria in all instances. The critical stage was found to last from the appearance of the primitive streak until the beginning formation of the notochord and neural plate. Byerly [202] described in somewhat older embryos as suffocation effect large blood vessels dilated into sinuses of excessive dimensions and death of the tissues in the deeper layers of the germ. I have seen the same abnormalities as "spontaneous" malformations in eggs which had not intentionally or knowingly been subjected to anoxia, which suggests anoxia as a natural cause of malformations. Anoxia due to failure of extraembryonic blood circulation to develop properly is thought to be responsible for some of the malformations and their peculiar lateral distribution in prothanic homozygous Creeper chick embryos.[46] The left eye, which is commonly more affected than the right, is turned away from the surface of the blastoderm by the normal rotation of the head and thus receives less oxygen by diffusion while the development of circulation lags. Transplantation experiments have shown that the primordia of the right and left eyes of these embryos are potentially equal.[203] Experimental obstruction of the extraembryonic circulation in genetically normal embryos leads to similar malformations with severer manifestations on the left side of the head, whereas an increase of the oxygen tension attenuates the defects of the left eye in prothanic Creeper embryos.[46] A subsequent examination of normal chick embryos incubated under favorable conditions showed that the left eye lags temporarily in the majority of them.[18] This asymmetry is present only during a short period beginning when the head turns to the side, and ending when an effective circulation is established. This is well in accord with the assumption of transitory anoxia of the left eye. A great preponderance of malformations of the left eye exists not only in Creeper embryos but also among sporadic [204] and selenium-induced malformations.[49] It may be assumed that temporary anoxia is a contributing factor which decreases the resistance of the left eye to other teratogenic agents.[18]

It is obvious that anoxia cannot be of great importance as a teratogenic factor in mammals. One can imagine that disturbances of oxygen supply occur before nidation in the uterus, or before the beginning of embryonic circulation, but this has not been observed. In later stages certain poisons may interfere with the metabolism of oxygen. A pertinent case of carbon monoxide poisoning of a pregnant woman has been described,[205] in which the mother recovered, while

202. Byerly, T. C.: Anat. Rec. **32**:249, 1926.

203. Gayer, K.: J. Exper. Zool. **89**:103, 1942.

204. (*a*) Gruenwald.[18] (*b*) Landauer, W.: Anat. Rec. **86**:365, 1943.

205. Maresch, R.: Wien. med. Wchnschr. **79**:454, 1929.

the child, which was born thirteen days after the poisoning and lived nine days, showed severest softening of the thalami and the corpora striata. In guinea pigs, anoxia preceding delivery produces severe cerebral damage.[206] In man, temporary anoxia of the older fetus stimulates respiratory movements, and may endanger the fetus by causing aspiration of amniotic fluid containing sebaceous material, cornified cells or meconium.[207] Damage of the brain similar to that observed in the aforementioned animal experiments may cause mental deficiency.[208]

Abnormal Temperature.—This environmental factor has no known teratologic importance in man and other mammals. Several authors have examined the influence of an abnormal incubation temperature in chick embryos and found various structural defects.[209] In the same species the expression of some hereditary malformations has been modified by a lowered temperature, as was mentioned.[210]

Petersen [211] has reported studies of the influence of various climatic factors on the incidence of malformations in man.

Infection and Inflammation.—The question whether maldevelopment may be caused by infection and subsequent inflammation leads to the frontiers of what is commonly considered to be maldevelopment as opposed to disease. That no strict borderline exists between these two divisions of the field of pathology, has been emphasized in the introduction.

The possible role of inflammation as a cause of abnormal development has been much discussed with respect to malformations of the heart. In the past it was believed that many of these malformations were due to embryonic endocarditis, but this explanation is no longer accepted for the great majority of cases. Gross [212] goes so far as to

206. (*a*) Windle, W. F.; Becker, R. F., and Weil, A.: J. Neuropath. & Exper. Neurol. **3**:224, 1944. (*b*) Windle, W. F., in Harvey Lectures, Lancaster, Pa., Science Press, 1945, vol. 40, p. 236.

207. (*a*) Farber, S., and Sweet, L. K.: Am. J. Dis. Child. **42**:1372, 1931. (*b*) Helwig, F. C.: Am. J. Obst. & Gynec. **26**:849, 1933. (*c*) Snyder, F. F., and Rosenfeld, M.: ibid. **36**:363, 1938. (*d*) Warwick, M.: New York State J. Med. **37**:2075, 1937.

208. Schreiber, F.: J. A. M. A. **111**:1263, 1938. Benda, C. E.: Am. J. Psychiat. **97**:1135, 1941. Clifford, S. H.: J. Pediat. **18**:567, 1941. Lamm, S. S.: Am. J. Ment. Deficiency **48**:131, 1943. Raymond, C. S.: ibid. **49**:8, 1944. Benda, C. E.: Medicine **24**:71, 1945.

209. Danforth, C. H.: Proc. Soc. Exper. Biol. & Med. **30**:143, 1932. Romanoff, A. L.: Poultry Sc. **15**:311, 1936. Smith, L. E. W.: Arch. Path. **28**: 422, 1939.

210. Sturkie.[52] Landauer.[51]

211. Petersen, W. F.: Am. J. Obst. & Gynec. **28**:443, 1934; Am. J. Orthodontics **27**:179, 1941.

212. Gross, P.: Arch. Path. **31**:163, 1941.

conclude from his own case and a review of the literature that there is no proved instance of fetal endocarditis, and others agree with him.[213] On the other hand, several authors[214] have described cases of acute endocarditis of undoubtedly prenatal origin. I have seen severe endocarditis of the tricuspid valve in an infant less than 1 day old.[215] These cases do not bear directly on the question of the inflammatory origin of cardiac malformations, as they concern late stages of intrauterine life. However, if one considers the relatively extremely small number of early embryos which are adequately examined (i.e. in serial sections), one must realize that failure to find acute endocarditis allows no conclusions. Fetal myocarditis has been found repeatedly.[216] Farber and Hubbard[216b] point out that of two groups into which congenital abnormalities of the heart may conveniently be divided, the one comprising gross departures from the normal developmental plan is not likely to be due to endocarditis. The other group includes hearts with normal septums and the typical relations of the large vessels and shows stenosis or atresia of valves, and these abnormalities are thought to be the effects of inflammation. I have found indications of a past inflammation, similar to those described by Farber and Hubbard, in the heart of a stillborn infant with atresia of the pulmonary ostium and in a newborn infant with stenosis of the mitral and aortic valves (cases unpublished).

The investigation of malformations of the heart and other organs occurring frequently in the infants of mothers who contracted rubella during the early months of pregnancy[217] may well contribute important information as soon as embryonic stages are examined. If the disease occurs during the first or second month of pregnancy, the inci-

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213. Weintraub, T., and Himmelfarb, A. J.: Bull. Johns Hopkins Hosp. **72**: 299, 1943.

214. Plaut, A., and Sharnoff, G.: Arch. Path. **20**:582, 1935. Püschel, E.: Arch. f. Kinderh. **114**:1, 1938. Plaut, A.: Am. J. Path. **15**:649, 1939. MacGregor, R. R., and McKendry, R.: Canad. M. A. J. **50**:433, 1944.

215. Dr. A. J. Gitlitz gave me the opportunity to see this specimen.

216. (*a*) Stoloff, E. G.: Am. J. Dis. Child. **36**:1204, 1928. (*b*) Farber, S., and Hubbard, J.: Am. J. M. Sc. **186**:705, 1933.

217. Swan, C.; Tostevin, A. L.; Moore, B.; Mayo, H., and Black, G. H. B.: M. J. Australia **2**:201, 1943. Swan, C.; Tostevin, A. L.; Mayo, A., and Black, G. H. B.: ibid. **1**:409, 1944. Erickson, C. A.: J. Pediat. **25**:281, 1944. Reese, A. B.: Am. J. Ophth. **27**:483, 1944. Gregg, N. M.: M. J. Australia **1**:313, 1945. Welch, L. S. V.: ibid. **1**:574, 1945. Long, J. C., and Danielson, R. W.: Arch. Ophth. **34**:24, 1945. Greenthal, R. M.: Arch. Pediat. **62**:53, 1945. Albaugh, C. H.: J. A. M. A. **129**:719, 1945. Carruthers, D. G.: M. J. Australia **1**:315, 1945. Prendergast, J. J.: Arch. Ophth. **35**:39, 1946. Guerry, D.: Am. J. Ophth. **29**:190, 1946. Goar, E. L. and Potts, C. R.: ibid. **29**:566, 1946. Swan, C., and Tostevin, A. L.: M. J. Australia **1**:645, 1946. Aycock, W. L., and Ingalls, T. H.: Am. J. M. Sc. **212**:366, 1946.

dence of malformations is nearly 100 per cent. Most commonly affected are the eyes (cataract, microphthalmia), the brain (mental retardation, microcephaly) and the heart. Only a few cases have as yet been studied at autopsy.[218] In contrast to most other authors, Fox and Bortin[219] conclude from their investigation of epidemics of rubella in Milwaukee that many pregnancies are unaffected. Conte, McCammon and Christie[220] sent 120 questionnaires to the mothers of infants with malformations of those types which may be caused by rubella. Of 61 mothers who answered, 5 gave a history of having had rubella during pregnancy. In view of the large number of other possible causes of the malformations in question, this is a significantly high incidence.

The developmental changes in fetal syphilis are too well known to be discussed here. Numerous reports deal with less common agents infecting the embryo—with virus,[221] bacteria[222] and protozoa.[223] Particularly tubercle bacilli have received a great deal of attention.[224] Localization of infectious organisms in the placenta is important in the mechanism of spreading of some of the diseases due to them, par-

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218. Swan, C.: J. Path. & Bact. **56**:289, 1944; Tr. Ophth. Soc. Australia **4**: 132, 1944.

219. Fox, M. J., and Bortin, M. M.: J. A. M. A. **130**:568, 1946.

220. Conte, W. R.; McCammon, C. S., and Christie, A.: Am. J. Dis. Child. **70**:301, 1945.

221. (*a*) Reuss, A., in Halban, J., and Seitz, L.: Biologie und Pathologie des Weibes, Berlin, Urban & Schwarzenberg, 1927, vol. 8, p. 2. (*b*) Lynch, F. W.: Arch. Dermat. & Syph. **26**:997, 1932. (*c*) Brody, H.: New York State J. Med. **41**:1256, 1941. (*d*) Woolpert, O. C.: Am. J. Path. **12**:141, 1936. Gallagher, F. W., and Woolpert, O. C.: J. Exper. Med. **72**:99, 1940. Goodpasture, E. W.: Science **95**:391, 1942. Goodpasture, E. W., and Anderson, K.: Am. J. Path. **18**:563, 1942.

222. Reuss.[221a] Wohlwill, F., and Bock, H. E.: Arch. f. Gynäk. **135**:211, 1929; Beitr. z. path. Anat. u. z. allg. Path. **85**:469, 1930. Burn, C. G.: Am. J. Path. **12**:341, 1936. Kelley, R. W.: Arch. Path. **28**:248, 1939. Diddle, A. W., and Stephens, R. L.: Am. J. Obst. & Gynec. **38**:300, 1939. Roback, H. N., and Kahler, H. F.: J. Nerv. & Ment. Dis. **94**:669, 1941. Price, S., and Chang, T.: Arch. Path. **41**:450, 1946.

223. (*a*) das Gupta, B. M.: Indian M. Gaz. **74**:397, 1939. (*b*) Paige, B. H.; Cowen, D., and Wolf, A.: Am. J. Dis. Child. **63**:474, 1942. (*c*) Koch, F.; Wolf, A.; Cowen, D., and Paige, B. H.: Arch. Ophth. **29**:1, 1943. (*d*) Zuelzer, W. W.: Arch. Path. **38**:1, 1944. (*e*) Pratt-Thomas, H. R., and Cannon, W. M.: Am. J. Path. **22**:779, 1946.

224. Whitman, R. C., and Greene, L. W.: Arch. Int. Med. **29**:261, 1922. Schmorl, G., in Engel, S., and Pirquet, C.: Handbuch der Kindertuberkulose, Leipzig, George Thieme, 1930, vol. 1, p. 137. Siegel, M.: Am. Rev. Tuberc. **29**:297, 1934. Siegel, M., and Singer, B.: Am. J. Dis. Child. **50**:636, 1935. Conrad, C. A.: South. M. J. **32**:169, 1939. Schaefer, G.: Quart. Bull., Sea View Hosp. **4**:457, 1939. Schwartz, R., and Gerhart, O.: Compt. rend. Soc. de biol. **129**:1095, 1939. Schenck zu Schweinsberg, H. G.: Monatschr. f. Kinderh. **88**:145, 1941. Loewenstein, E.: Am. Rev. Tuberc. **51**:225, 1945.

ticularly tuberculosis. As far as the investigators know, developmental disorders are but rarely caused by these infections. However, the example of rubella shows how unsuspected correlations between embryonic infection and malformation may become apparent and their medical importance appreciated once they are brought to attention. Cellular inflammatory reaction to infection in embryonic tissues has been examined in experimental and clinical material.[225]

COMMENT

The knowledge of the causes of malformations reviewed in the preceding pages is based largely on experiments. Part of it, being based on experiments with lower vertebrates, does not apply directly to man and other mammals with their excellent protection of the embryo. It has been reviewed not only in order to illustrate general principles of teratology but also because the resulting malformations are often comparable in more or less detail with mammalian ones. The development of the malformation in an experimental animal shows how a similar condition in man may have arisen. This comparison cannot offer more than a suggestion, since it is known that different mechanisms may lead to similar final results. Incidental findings of a few early stages in man may then support or refute the comparison. Moreover, the time of action may be more important than the exact nature of a teratogenic agent, as will presently be discussed. Thus the action of agents which in themselves do not occur in man and other mammals may be duplicated by others which exert the same influence (e.g., inhibition of development) at a comparable time and place in the embryo.

It has repeatedly been postulated that the action of detrimental agents on development depends to a considerable degree on intrinsic circumstances in the embryo. As an example, the previously mentioned temporary anoxia of the left side of the head of the chick embryo may be recalled. It not only produces a transitory lag in the normal development of the left eye but is presumably the cause of the marked preponderance of sporadic malformations of the left eye of the chick embryos.[18] More often a rapid rate of growth and development has been regarded as a predisposing factor,[18] and it is on this basis that investigators have explained how nonspecific detrimental factors acting on the entire embryo during limited periods may produce well defined

225. Wohlwill, F., and Bock, H. E.: Virchows Arch. f. path. Anat. **291**:864, 1933. Lillie, R. D.: Pub. Health Rep. **50**:1498, 1935. Goldsworthy, N. E., and Moppett, W.: J. Path. & Bact. **41**:529, 1935. Goodpasture, E. W., and Anderson, K.: Am. J. Path. **13**:149, 1937. Buddingh, G. J., and Polk, A. D.: J. Exper. Med. **70**:485 and 499, 1939. Canat, E. H., and Opie, E. L.: Am. J. Path. **19**:385, 1943.

malformations only in certain organs. Child [226] and his followers have developed the concept of gradients of vital activities in developing organisms and have shown how these gradients influence normal and abnormal development. Thus Hyman [227] has discussed the influence of high rates of growth at given points on the effects of injurious agents. The defect produced is more severe in a part whose rate of growth is higher than those of others exposed to the same agent. Up to a certain limit, this may be compensated by a great ability of the rapidly growing part to make restitution of itself. Beyond that limit, the permanent damage will be greater in a part with a high growth rate than elsewhere. Thus a pattern of sensitivity exists in the developing organism at a given stage which may be responsible for identical reactions to widely differing teratogenic agents, genetic and environmental. Not only can different abnormal genes produce the same malformation,[54] but exact phenocopies [228] of hereditary abnormalities may be produced by agents affecting the tissues of the individual itself and not its genetic constitution.[229] The concept has evolved that at least part of the teratogenic genes affect the embryo in a nonspecific manner at a constant stage of embryonic development. This agrees well with recent work suggesting that much if not all of gene action affects metabolic processes.[12] It has been pointed out that phenocopies have been produced only of those hereditary traits which are assumed to be caused by a change in the relative rates of developmental processes (which are of greater importance in the production of malformations). On the other hand, no phenocopies are on record of traits which interfere with definite steps of chemical synthesis.[230] Perhaps this is so because the former act but once, whereas the latter act throughout life and cannot very well be imitated by extrinsic agents. The assumption of an action of genes on metabolism is supported by several previously mentioned observations. Lowering the incubation temperature and thus the rate of growth of the embryo at a given time can reduce its reaction to gene action and diminish the expression of certain hereditary traits in the chick.[210] The cumulative effect of the hereditary Creeper factor and selenium poisoning in the chick embryo has been explained by the assumption that both interfere in a similar manner with metabolic activity.[49]

226. Child, C. M.: Am. Naturalist **58**:237, 1924; Patterns and Problems of Development, Chicago, University of Chicago Press, 1941.

227. Hyman, L. H.: Biol. Bull. **40**:32, 1921.

228. A phenocopy is an abnormality closely resembling a certain hereditary trait, but produced by an influence on the individual itself, and not hereditary.

229. (*a*) Cairns.[46] (*b*) Kaven, A.: Ztschr. f. menschl. Vererb.- u. Konstitutionslehre **22**:247, 1938; footnote 92.

230. Goldschmidt, R. B.: J. Exper. Zool. **100**:193, 1945.

It is apparent that malformations are the effect of a variety of intrinsic and extrinsic, synergistic or antagonistic agents. Many of these act in a nonspecific manner and may be replaced by others with similar action without changing the final result.

In the preceding pages one may find several instances of discrepancies in the results obtained by different workers with the same agent. This may be due to differences in the technic of the experiment or in the genetic constitution of the animals used. It is obvious from what has just been said that slight variations of the strength of the agent and particularly of the timing of its action may change the results fundamentally. The interaction of genetic and environmental factors in the production of malformations has been referred to on several occasions, and this may explain variations in the results of experiments with different strains of animals.

There is a method of approach to malformations and their causative agents which has not yet been mentioned in this review, namely, statistics. It is quite possible that in the future many questions of the action of hereditary or environmental influences on the developing organism will be answered by statistical evaluation of large groups of cases. Particularly concerning human maldevelopment, results might be obtained which would otherwise not be available, as experimental methods cannot be used. A detailed study of a large number of human cases correlated with information on various aspects of environment and other factors has been made by Murphy.[11] The only consistent result so far obtained in several independent statistical investigations is a striking difference between white persons and Negroes in the over-all incidence of malformations; the incidence is higher in white persons.[231]

231. Murphy.[11] Potter, E. L.: J. A. M. A. **115**:996, 1940. Gruenwald, P.: Illinois M. J. **79**:55, 1941.

(To Be Continued)

Notes and News

State Toxicologic Service.—A toxicologic service has been organized in the department of pharmacology and toxicology of the University of Illinois College of Medicine, of Chicago, under the direction of C. C. Pfeiffer. This service will be available to the offices of state's attorney and coroner in all the counties of Illinois. Similar services are used in Michigan, Indiana and Wisconsin.

Careers in Academic Medicine.—An opportunity to start a career in academic medicine is offered by the John and Mary R. Markle Foundation, 14 Wall Street, New York 5, to young scientists with the necessary training to hold regular faculty appointment and to conduct research. The new program of postfellowship grants will be conducted in cooperation with accredited medical schools of the United States and Canada. Grants of $25,000, to be paid to the cooperating school at the rate of $5,000 annually toward the support of each successful candidate or his research or both, will be available, beginning with the academic year 1948-1949. If the plan proves successful, the foundation will appropriate a total of $1,250,000 payable to the schools by 1953. Candidates will be recommended by medical schools, and recommendations will be limited to young men and women who show a particularly strong interest in research and in teaching in any of the clinical or preclinical sciences or in the sciences basic to medicine. They will have had training in some special field or combination of fields to qualify them to receive a regular faculty appointment and to conduct original research. The final choice of "Scholars in Medical Science" will be made, on the basis of the schools' recommendations, by regional committees appointed by the foundation. About fifty will receive appointments during the five year period. The school will determine salary and academic rank, encourage research by setting reasonable limits on nonresearch activities, provide laboratory facilities and, if necessary, make a financial contribution toward support of the scholar's work. The program is the result of a survey of medical research and education recently made by the foundation, which shows that while there are scholarships and other forms of financial aid for the student in the course of his scientific training, and while there are funds available to the scientist once his name is made, there are few sources of help at the beginning of the career of the man who chooses academic medicine. Persons interested in being considered as candidates are referred to deans of accredited medical schools for further information.

Maternal Infections and Congenital Malformations.—The American Academy of Pediatrics and the National Society for the Prevention of Blindness have organized a nationwide survey to collect data which may establish a relationship between infection in the expectant mother and the occurrence of congenital defects in the offspring. Measles, chickenpox, mumps and influenza are examples of maternal infections. Physicians knowing of such cases are asked to register them with Dr. Herbert C. Miller, professor of pediatrics, University of Kansas School of Medicine, Kansas City, Kan.

The World Federation of Pathologists.—The European Association of Clinical Pathologists has reconstituted itself as a world federation of societies devoted to clinical pathology. The federation aims (1) to develop clinical pathology,

which is the application of pathology and allied sciences to medicine, (2) to have regard to the scientific and professional status of those engaged in its practice, and (3) to foster international amity. A committee will call a meeting of delegates from as many interested associations as possible next November in Paris, during the meeting of the French Société de Biologie Clinique, to formulate a constitution and elect officers. The federation invites societies devoted to clinical pathology to communicate with the secretaries—Dr. W. H. McMennemy, pathologic department, Royal Infirmary, Worcester, England, or Dr. J. Ungar, Glaxo Laboratories, Ltd., Greenford, Middlesex, England. Medical practitioners or university graduates practicing clinical pathology who are members of the constituent bodies will thereby become members of the federation. Membership will be available also to those who are eligible but do not belong to a constituent body. The federation proposes to hold a conference at least once every three years at the same time and place as the summer meeting of one of its constituent bodies. It hopes that the first meeting will take place in the summer of 1948.

CORRECTION

An error was made in numbering the legends for figures 1 and 2 in the article by Major Welland A. Hause and Captain Gunnard J. Antell entitled "Arteriosclerosis in Infancy," which appeared in the July issue (ARCH. PATH. **44**:82, 1947). Under figure 1 the legend designated *B* should be *A;* that designated *A* should be under figure 2, on page 85. The legend under figure 2 is the legend for 1 *B*.

The magnification of figure 2 is × 235, instead of × 70.

Books Received

A HANDBOOK FOR THE DIAGNOSIS OF CANCER OF THE UTERUS BY THE USE OF VAGINAL SMEARS. By Olive Gates, M.D., pathologist, Massachusetts State Tumor Diagnosis Service; assistant pathologist, Pondville Hospital (Massachusetts Department of Public Health), and Shields Warren, M.D., assistant professor of pathology, Harvard Medical School; pathologist, New England Deaconess and New England Baptist hospitals; reserve consultant in pathology to the United States Navy Bureau of Medicine and Surgery, Captain (M.C.) U.S.N.R. With a Foreword by George N. Papanicolaou, M.D., Ph.D., associate professor, Department of Anatomy, and research associate, Department of Obstetrics and Gynecology, Cornell University Medical College and New York Hospital. Pp. 182, illustrated. Cambridge, Mass.: Harvard University Press, 1947.

This book should find a ready audience among physicians who, in increasing numbers, are becoming interested in the vaginal smear used as an adjunct of the existing means of diagnosing cancer of the uterine corpus and cervix. It begins with an attempt to evaluate the usefulness of the procedure and subsequently gives the methods of preparing the smear, a brief description of the normal histologic pattern of the female reproductive organs and a discussion of the criteria warranting a diagnosis of cancer. It concludes with 50 excellent plates, each made up of from 2 to 6 photomicrographs showing the representative types of cells found in the vaginal smear.

The value of the vaginal smear as a means of making a diagnosis of cancer earlier than would otherwise be possible has not yet been established, and, as the authors point out, a conclusion can be reached only after large numbers of careful observations have been made and adequately evaluated. Two tables presented by the authors are especially interesting. One concerns 6,265 cases in which vaginal smears had been made. Among the 475 cases in which carcinoma of the uterus was found, no symptoms were present in 8, and smears were positive in 8, although the first biopsies gave negative results. The other table includes 5,583 cases, 490 of which were proved to be cases of cancer. In this group 120 erroneous diagnoses were made on vaginal smears; in 63 of these a false positive diagnosis was made, and in 57 the diagnosis was not made even though cancer was eventually proved to be present.

The authors point out the difficulties attendant on the attempt to make a diagnosis of cancer from one or a few cells and refer to the time-tested belief that the general structure of tissue, as well as the individual cell pattern, must be taken into account in arriving at a conclusion. They stress the time-consuming nature of the examinations and state that a minimum of twenty minutes should be allowed for each smear unless an obvious carcinoma is present and that at times an adequate examination requires an hour. They believe that an untrained person of average aptitude who is studying for two hours daily under good supervision will require four months to a year to attain proficiency in diagnosis.

The descriptions of the various types of normal and abnormal cells found in vaginal smears are detailed and should be of much help to the inexperienced observer. The photomicrographs are well reproduced and will be found of value. It is always difficult, however, to obtain an adequate idea of cell structure from photographs, and, excellent as these are, the average observer will find many cells in actual smears that he will be unable to identify by use of the plates alone.

It is refreshing to find a book devoted to a relatively new subject which so dispassionately presents and analyzes the material. It is carefully planned, well written and comprehensive in scope. It could be read with profit by all physicians interested in the diagnosis of cancer of the uterus.

CANCER: DIAGNOSIS, TREATMENT AND PROGNOSIS. By Lauren V. Ackerman, M.D., pathologist to the Ellis Fischel State Cancer Hospital and assistant professor of pathology at Washington University School of Medicine, St. Louis, and Juan A. del Regato, M.D., radiotherapeutist to the Ellis Fischel State Cancer Hospital and formerly assistant to the Radium Institute of the University of Paris. Pp. 1,115, with 745 test illustrations and 42 color reproductions. Price $20. St. Louis: C. V. Mosby Company, 1947.

In spite of the progress made in surgical and radiation therapy during the last twenty-five years, the over-all picture allows little improvement of results of cancer therapy to be recognized. Only a statistically negligible minority of patients with curable cancers have the benefit of adequate therapy without avoidable delay. One of the main reasons for this situation is the lack of coordination of teaching of the different phases of cancer—pathology, biology, diagnosis, therapy—in medical schools, so that "even the better than average interne frequently lacks adequate understanding of malignant disease" (National Advisory Cancer Council, 1946).

The book by Ackerman and Regato is eminently fitted to help fill this gap. This book is to this reviewer's knowledge the most comprehensive, most reliable and, for practical purposes, best balanced presentation available for the use of the undergraduate student, the general practitioner and the specialist.

The smaller general part of the book contains chapters entitled "Pathology of Cancer," "Surgery of Cancer" and "Radiotherapy of Cancer." Each one of these gives a synopsis of the clinically pertinent points which in practice frequently are not appreciated in their true importance. These chapters are preceded by a short introduction, in which some statistical facts are discussed, and a chapter on cancer research contributed by Michael B. Shimkin, of the National Cancer Institute, which gives an instructive summary of the trends and accomplishments noted in the research field to date.

While the general chapters contain many most useful practical suggestions, the selection of which is obviously derived from the daily experience of the authors, their composition is somewhat haphazard—at times too specialized for the general reader, yet not detailed enough for the specialist, as for instance some physical and technical discussions of radiation therapy. The chapter entitled "Pathology of Cancer" is probably the one best integrated in the general context of the book. Remarks about the technic of obtaining a specimen for biopsy and the necessity of submitting sufficient data for its evaluation are much to the point. The responsibility of the pathologist and the limitations of his services are briefly but precisely discussed. The conservative attitude of the authors with reference to the clinical importance of tumor grading is most satisfying.

The second part of the book is devoted to the cancers of different organs in detail. The length of the chapters varies greatly and is not commensurate with the incidence of the tumor under consideration. It was obviously determined by the practical necessity of distributing greater knowledge of some less generally known aspects of the more curable forms of cancer, and the more important recent developments have received priority on space. Likewise is there a marked difference in the quality and the originality of different chapters, naturally depending on difference in the personal experience and particular interest of the authors.

The outstanding chapter, of almost 300 pages, "Cancer of the Respiratory System and Upper Digestive Tract," is based on the unusually great personal experience of the authors in this field. In it they discuss in detail the forms of cancer of the sinuses, the oral cavity, the lip, the pharynx and the larynx. This field, as daily experience teaches, is still quite confusing to many physicians' minds as far as precise diagnosis and understanding of the relative indications of surgical and radiation therapy and the possible accomplishments are concerned. This chapter represents, probably, the most comprehensive and authentic presentation of this particular field available, and if it could be published as an independent monograph for the use of the respective specialists, that would be highly desirable. A careful study of the detailed discussion of the types of tumors encountered from the point of gross and microscopic pathology and exact location as well as from the point of accessibility to surgical treatment or irradiation should greatly help to clarify the situation, and this in turn undoubtedly would lead to an increased rate of cure of these tumors, the curability of which is high if they are recognized early and treated properly.

Two other excellent chapters are those on cancer of the skin and cancer of the female genital organs. In the discussion of treatment of cutaneous cancers the emphasis on the care with which treatment—surgical and radiologic—is to be adjusted to the individual requirements, dependent on location and extent of the disease, with abandonment of any routine procedure applied to all cases, is particularly to the point and will help to prevent some of the failures which may make a curable cancer incurable.

In the discussion of cancers of the female genital organs, the important portion on carcinoma of the cervix is precise and well balanced. In spite of the by now fairly well standardized situation, much can be learned from careful study of this chapter. Particularly, the detailed description of the clinical staging (supported by excellent illustrations of the types of lesions encountered in the different stages) will help to clarify a still quite prevalent confusion which was partly caused by the change of the League of Nations classification in 1937 and thus facilitate an intelligent appreciation of results of treatment. The practical suggestions concerning certain details of the pelvic examination of the cancerous patient as contrasted with the routine gynecologic examination (the examination with both the right and the left hand, the technic of the examination of the rectovaginal septum, examination with the speculum preceding detailed palpation) are only a few of the many points which demonstrate the meticulous attention of the authors to details that are important in practice. Likewise does such attention to details of the individualization of treatment lead the authors to refute the myth of radioresistant cancers of the cervix by the statement, "No carcinoma arising from the cervix is radioresistant. Even adenocarcinomas, which for many years were judged less amenable to radiotherapy, have long been recognized as radiosensitive and radiocurable." The now again prevalent and frequently exaggerated controversy of radiotherapy against surgery is reduced to its true proportions by the observation that only 10 per cent of the total number of patients would be suitable for surgical treatment.

The other common forms of cancers of the female genital organs are adequately discussed, but one misses a discussion of endometriosis, which, particularly in its stromal variety, may be important for differential diagnosis. Most of the previously diagnosed "sarcomas" of the uterus probably belong in this group and a more general knowledge of this curable disease would be desirable.

In contrast to these outstanding and original chapters, one finds some chapters dealing with the more commonly known varieties of cancer in a more conventional

way. Still the information given is based on a careful evaluation of the particular experiences either of the authors themselves or of other workers. Considerable, on the whole undisputed, information is thus presented under the headings: "Tumors of the Thyroid Gland"; "Tumors of the Mediastinum"; "Cancer of the Digestive Tract"; "Cancer of the Genito-Urinary Tract"; "Cancer of the Male Genital Organs"; "Tumors of the Suprarenal Gland"; "Cancer of the Mammary Gland"; "Malignant Tumors of Bone"; "Sarcomas of the Soft Tissues."

Some short chapters on cancer of the eye, Hodgkin's disease and leukemia conclude the text.

To each chapter is appended a bibliography of the sources cited in the text.

Each one of the special chapters discusses anatomy, incidence and etiology, pathology, clinical evolution, diagnosis, treatment and prognosis. While such rigid organization could easily have led to an academic enumeration of facts, it is the educationally outstanding and truly unique feature of this book that the authors succeeded in intelligently coordinating anatomy, pathology and clinic. Accordingly, the emphasis varies considerably in different chapters, depending on the practical importance of the different phases. One of the most important features is the detailed discussion of the lymphatic channels of each organ, the precise knowledge of which is so important for rational management. Excellent sketches facilitate understanding of this subject.

Every phase of the discussion is profusely illustrated. The most instructive illustrations are the excellent sketches prepared for the elucidation of the text. The photographic illustrations are in general adequate for the demonstration of the point in question, though by no means technically brilliant. While most of the illustrations add to understanding, some—like those of far advanced, hopeless lesions—represent, rather, museum pieces without didactic value. These as well as all the color plates should have been omitted. The color plates are poorly executed, and none of them adds to the educational value. Their elimination may reduce the price of the book and thus increase its distribution and usefulness.

The enormous amount of material is presented in a detached and unbiased way. Everywhere one can recognize the critical evaluation of the authors' own matured experiences, with proper integration and critical evaluation of the opinions and experiences of other workers. Although the book is written by a pathologist and a radiologist, there is nowhere undue emphasis on the possibilities and accomplishments of radiation therapy. In a way it is regrettable that no surgeon has cooperated —not that this would have improved the presentation or would have changed the statements as to the relative place of irradiation and surgical procedure, which are most conservative—but it might have given more weight to the conclusions in the minds of those who still do not fully appreciate the place of irradiation for certain forms of cancer. While the book is not primarily meant for the cancer specialist—it contains little technical detail—the wealth of the authors' experiences and their intelligent and critical evaluations make fascinating reading for the experienced specialist in the field, who as a gourmet will enjoy many of the intellectual delicacies. As is to be expected with a book of this scope, there will be minor disagreements respecting some of the authors' opinions. As a whole, however, the book represents one consistent unit which reflects the prolonged close cooperation of the authors and their profound understanding of the problems involved. This work in itself again demonstrates the value of such close cooperation in the field of cancer therapy. If it is used as much as it deserves, it will represent one of the most realistic contributions of the last decade to the fight against cancer.

THE AMERICAN ILLUSTRATED MEDICAL DICTIONARY: A COMPLETE DICTIONARY OF THE TERMS USED IN MEDICINE, SURGERY, DENTISTRY, PHARMACY, CHEMISTRY, NURSING, VETERINARY SCIENCE, BIOLOGY, MEDICAL BIOGRAPHY, ETC., WITH THE PRONUNCIATION, DERIVATION AND DEFINITION. By W. A. Newman Dorland, A.M., M.D., Lieutenant Colonel, Medical Reserve Corps, United States Army, member of the Committee on Nomenclature and Classification of Diseases of the American Medical Association; editor of the "American Pocket Medical Dictionary." Twenty-first edition. Price, $8 without thumb index; $8.50 with the thumb index. Pp. 1660, with 880 illustrations, including 233 portraits. Philadelphia and London: W. B. Saunders Company, 1947.

The first edition was published in 1900. The succeeding editions have represented well the steady growth of the medical vocabulary, and the present edition appears to cover as completely as can be expected the manifold additions to the vocabulary during the war and since. The standards of factual and typographic accuracy are well maintained. Many names of discarded proprietary medicines have been omitted, also some portraits. The only new portrait is that of Howard T. Ricketts, pathologist and pioneer investigator of rickettsial infections. The number of pages is about the same as in the previous edition. The definitions relating to cancer should be critically revised, beginning with the word "cancer" itself, which is defined as "any malignant tumor made up chiefly of epithelial cells. See carcinoma." In current usage, popular as well as technical, the term "cancer" includes sarcoma as well as carcinoma. "Alveolar carcinoma" and "mammary sarcoma" are examples of other cancer terms needing corrected definitions.

LE PROBLÈME DU CANCER ET SON ÉVOLUTION RECENTE (CANCER ET NEURO-ERGONOLOGIE). By Michel Mosinger, Institut d'Anatomie Pathologique de l'Université de Coïmbra (Portugal). Preface Prof. Gustave Roussy. Pp. 664, with 184 illustrations. Price, 1,000 francs. Paris: Masson & Cie, 1947.

The author reports a number of facts bearing on the etiology of cancer. The dominant idea is that the neurovegetative system plays an important direct or indirect role in the pathogeny of cancer. The cancerization of the normal cell is considered as the result of progressive phases: hyperplasia—benign tumor—cancer. The author presents the hypothesis that cancerization is essentially due to a disruption of the normal equilibrium of intracellular and extracellular substances. He distinguishes between carcinogens and carcinoinhibitors.

This appears to be a special issue (1944) of the Portugese journal *Arquivos de Anatomia Pathologica*, etc., presented in book form. The author is a French biologist who, at the time of writing, is director of the Institute of Pathologic Anatomy of Coimbra, Portugal. This probably explains the peculiar mixture of French with Portuguese text. (The latter language appears abruptly in page 30 and is kept through page 49, where it vanishes.)

The author reports his own work on 320 guinea pigs treated by injections of estradiol dipropionate and other products. As much as 243 pages are devoted to tedious enumeration of carcinogenic compounds with profuse text references, the majority of which are not listed in the bibliography. Sixty pages are devoted to a rather light description of multiple publications touching numerous subjects. The photographs are an example of the undesirable habit of using such means to balance a monotonous text; they are usually of poor quality.

The reader will be attracted by a section which purports to outline the perspective and organization that should be given to future research in cancer, but no one would escape the empty feeling that is left after reading it.

COLLECTED PAPERS OF THE MAYO CLINIC AND THE MAYO FOUNDATION. Edited by Richard M. Hewitt, M.D.; A. B. Nevling, M.D.; John R. Miner, Sc.D.; James B. Eckman, Ph.D.; Katharine Smith, B.A.; Carl M. Gambill, M.D., M.P.H., and Elizabeth L. Skafte, B.A. Volume 38, 1946. Pp. 915, with 149 illustrations. Price, $12.50. Philadelphia and London: W. B. Saunders Company, 1947.

REPORT OF THE HENRY PHIPPS INSTITUTE OF THE UNIVERSITY OF PENNSYLVANIA FOR THE PERIOD 1944-1946. Pp. 24. Philadelphia: Henry Phipps Institute, 1947.

INTERNAL MEDICINE IN GENERAL PRACTICE. By Robert Pratt McCombs, B.S., M.D., assistant professor of medicine and director of post-graduate teaching, Tufts College Medical School; senior attending physician, Joseph H. Pratt Diagnostic Hospital; specialist certified by the American Board of Internal Medicine. Second edition. Pp. 741, with 122 illustrations. Price $8. Philadelphia and London: W. B. Saunders Company, 1947.

ANNUAL REPORT FOR THE YEAR ENDED MAY 31, 1947. Issued by the National Foundation for Infantile Paralysis. Publication no. 68. Pp. 86, illustrated. New York: The National Foundation for Infantile Paralysis, 1947.